Pathogenic mechanisms of chronic infections.

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“Perhaps, as biologists, we may all agree on one aspect of nature, namely, its exceeding variety. Even a parasite may choose the course of manifest destiny and find aggressiveness more attractive and more valuable than an existence of peace and symbiosis.”

Gordon H. Ball, 1943. Parasitism and evolution. Am. Nat. 77: 345–364.

Contents

Abstract

Staphylococcus aureus (*S. aureus*) is a very versatile opportunistic human pathogen. Besides an asymptomatic colonisation of humans, it can cause a multiplicity of infections, which vary in severity. By investigating a possible connection between certain pathologies and bacterial phenotypes, general and specific infection patterns are supposed to be identified. Therefore, a strain collection based on four *S. aureus* induced infections and colonisation, with 10-12 strains per group, was established. Staphylococcal strains were isolated from sepsis, haematogenous osteomyelitis, prosthesis infections and from the nasal area of healthy people. Together with the acquisition of selected patient characteristics, strains were characterised genotypically and by their phenotypic expression of virulence factors. Moreover, the bacterial interaction with osteoblasts was analysed in functional assays that determined invasiveness and cytotoxicity. Selected bacterial isolates were examined for their ability to persist within bone cells and their performance in a murine sepsis / osteomyelitis model.

Against expectations, a pathology specific bacterial pattern could not be identified. The analysis of patient characteristics indicates an important role of the host's health status for the course of the infection. However, in all isolate groups, the values of the individual strains were distributed in a broad spectrum. The application of correlation analysis helped to identify two distinct infection strategies. It revealed the opposing nature of invasion and cytotoxicity as low cytotoxic strains tend to be high invasive and vice versa. Even though *S. aureus* was shown to be able to invade, persist within cells, and to form small colony variants (SCVs) independently of its cytotoxicity, low cytotoxic strains exhibited higher persistence rates. This observation can possibly be attributed to the decreased production of toxins and the reduced activation of the host immune system.

In conclusion, the obtained results highlight the importance of the consideration of phenotypic diversity in pathogens with special regard to apparently less dangerous variants, like low cytotoxic *S. aureus* strains. Although those strains are characterised by a low- toxin emission, they were shown to be superior persisters in infection models and to be a causative agent of life-threatening infections in patients. Consequently, strains with this phenotype are perfectly adapted for persistence and therefore for chronic infections, which are hard to cure and often recurring.

This work emphasises the influence of different bacterial survival strategies and the variance of interaction with the human host. The comprehensive understanding of host-pathogen interaction on an individual level is a mandatory prerequisite on the way to a personalised medicine.

Zusammenfassung

Staphylococcus aureus (*S. aureus*) ist ein sehr vielfältiges, opportunistisches Humanpathogen. Neben der asymptomatischen Kolonisierung von Menschen kann es eine Vielzahl von Infektionen auslösen, die in ihrer Schwere variieren. Durch die Untersuchung von möglichen Verbindungen zwischen bestimmten Pathologien und bakteriellen Phänotypen sollten allgemeine und spezielle Infektionsmuster identifiziert werden. Dafür wurde eine Stammkollektion, basierend auf vier *S. aureus* - induzierten Infektionen und Kolonisation mit 10-12 Stämmen pro Gruppe, etabliert. Die Staphylokokken-Stämme wurden von Sepsis, hämatogener Osteomyelitis, Protheseninfektion und aus dem Nasenraum von gesunden Probanden isoliert. Zusammen mit der Erhebung von ausgewählten Patientencharakteristika wurden die Stämme genotypisch und phänotypisch hinsichtlich ihrer Expression von Virulenzfaktoren charakterisiert. Weiterhin wurde die bakterielle Interaktion mit Osteoblasten in funktionellen Assays analysiert. Dadurch konnten die Invasivität und Zytotoxizität der Isolate quantifiziert werden. Ausgewählte *S. aureus* - Stämme wurden auf ihre Fähigkeit zur Persistenz in Knochenzellen und ihr Verhalten in einem murinen Sepsis-/Osteomyelitis-Modell hin untersucht.

Wider Erwarten konnte kein krankheitsspezifisches bakterielles Muster identifiziert werden. Die Analyse der Patientencharakteristika deutete den allgemeinen Gesundheitsstatus des Patienten als krankheitsentscheidendes Kriterium an. Allerdings waren die Isolate in allen Gruppen auf einem weiten Spektrum für die jeweiligen Eigenschaften verteilt. Die Verwendung von Korrelations-Analysen ermöglichte die Identifizierung von zwei distinkten Infektionsstrategien. Die Invasivität und Zytotoxizität wurden als zwei gegenläufige Konzepte erkannt, da niedrig zytotoxische Stämme für gewöhnlich hoch invasiv sind und umgekehrt. Obwohl der Grad der Zytotoxizität keinen generellen Einfluss auf die Fähigkeit zur Invasion, Persistenz und der Bildung von *Small Colony Variants* (SCVs) hat, zeigten niedrig zytotoxische Isolate höhere Persistenzraten. Diese Beobachtung ist möglicherweise auf die verminderte Toxinproduktion und die verringerte Aktivierung des Immunsystems zurückzuführen.

Zusammenfassend heben die gewonnen Ergebnisse die Bedeutung der phänotypischen Pathogenvielfalt hervor, mit besonderer Beachtung von scheinbar weniger gefährlichen Varianten, wie den niedrig zytotoxischen *S. aureus* - Stämmen. Obwohl diese Stämme durch eine geringere Toxinabgabe gekennzeichnet sind, konnte ihre überlegene Fähigkeit zur Persistenz im Infektionsmodell festgestellt und sie als Erreger von lebensbedrohlichen Infektionen in Patienten identifiziert werden. Infolgedessen sind Isolate dieses Phänotyps vollständig an eine

persistierende Lebensweise und damit chronische Infektionen angepasst. Diese Art von Infektionen sind oft schwer heilbar und durch eine hohe Rückfallrate gekennzeichnet.

Diese Arbeit hebt den Einfluss von verschiedenartigen bakteriellen Überlebensstrategien in Interaktion mit dem menschlichen Wirt hervor. Das umfassende Verständnis von Wirt-Pathogen-Interaktion auf einem individuellen Niveau ist zwingende Voraussetzung auf dem Weg zu einer personalisierten Medizin.

1. Introduction

S. aureus is a non-motile, gram-positive bacterium with the size of 0.5-1.5 µm. The species name describes the morphology, as the colonies are often golden (Latin *aureus* means "gold") and consist of spherical bacteria in grape-like clusters (Greek *staphylē* means "grape"; *kókkos* means "grain, seed, berry") as shown in Figure 1. As a gram-positive bacterium, *S. aureus* is coated with a thick cell wall that consists of numerous peptidoglycan layers which are interwoven with teichoic acids. Additionally, the majority of *S. aureus* possess a microcapsule that was shown to mediate resistance towards phagocytic clearance [1]. The endowment with catalase and coagulase were important diagnostic features in the past that helped to differentiate *S. aureus* from other genera and species.

Sir Alexander Ogston published the first description of *S. aureus* in 1880 and 1882 [2-4] where he identified *S. aureus* as the causative agent of purulent infections and sepsis. These first findings are today complemented by a variety of other diseases that can be attributed to infections with this versatile, well-adapted pathogen.

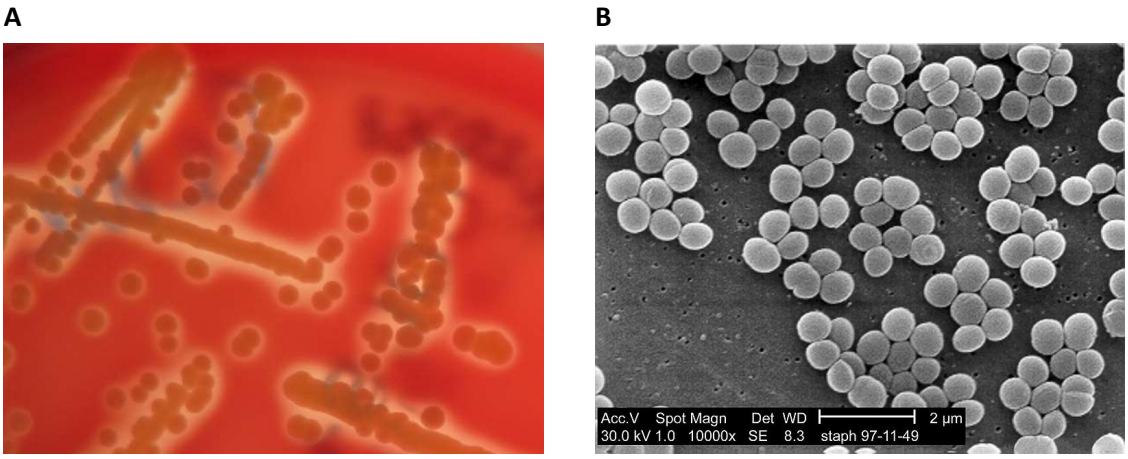


Figure 1 Morphology of *S. aureus*. A *S. aureus* forms yellow colonies with visible haemolytic clearance (strain dependent) on Columbia blood agar plate. **B** The scanning electron microscopy picture shows the clustering of *S. aureus* in grape-like structures. (Photo Credit: Janice Carr, Content Providers: CDC/ Matthew J. Arduino, DRPH; Janice Carr)

The introduction of antibiotics like penicillin or methicillin, resistant *S. aureus* appeared a few years later [5, 6]. The rising rates of methicillin-resistant *S. aureus* (MRSA), which mostly exhibited resistance to numerous other antibiotics, attracted broad public attention in the 1990s [7]. Nowadays the prevalence of MRSA is steadily decreasing in Europe [8]. It is still under debate if advanced prevention measures or replacement of MRSA by periodical prevalent MSSA strains is the reason for this development [9, 10].

1.1 An inconspicuous neighbour – *S. aureus* as commensal

The interaction between *S. aureus* and humans is very diverse. The bacterium is colonising 25-30% of all humans [11, 12] persistently without being eradicated by the host. Although the anterior nares are the most common site of colonisation [13], *S. aureus* can be isolated from throat, perineum, intestine, chest / abdomen, and axillae (s. Figure 2) which can be the exclusive colonisation location [14-16]. The decolonisation of *S. aureus* from the nose causes the vanishing from all other spots of colonisation which highlights the role of the nose as primary niche for staphylococcal colonisation [17, 18]. If expectant mothers are colonised with *S. aureus*, they will probably transmit their bacterial strain on their infant during or after birth [19-21]. Later in life, the main transmission route is from hand to hand or from surfaces to hand [22]. The habit of nose picking promotes the transfer of staphylococci from hand to nose thus encouraging colonisation [23]. If the *S. aureus* carrier is a heavy disperser and suffers from an infection of the upper airways, even an airborne transmission is possible [22, 24], however negligible, compared to the fact that most of those bacteria are transferred via direct contact [25].

Traditionally, three different types of time-defined carriage are differentiated: persistent, intermittent, and non-carriage [14]. The minority of people is permanently colonised with a single *S. aureus* strain (20%), an equal amount is never colonised, and the majority of people belongs to the group of intermittent carriers (60%) where strains of varying clonality are detected at times [26]. Why some people are never colonised, not even as new-borns [27], is not fully understood yet [28]. The highest carriage frequency is observed in children where almost every second child was colonised [29] which could probably be attributed to the elevated rates of intermittent colonisation in this group [30]. Intermittent carriers are usually colonised by changing staphylococcal strains [31, 32]. If the same is true for persistent carriers is currently under debate [33] and must be clarified by future research. Moreover, there are hints for an age-related clonal pattern [29] which corroborates the dynamic aspect of colonisation. If members of a family or household are carrying a commensal *S. aureus,* they most likely share strains from the same clonal background [19, 33, 34] indicating a horizontal route of transmission. A similar way of transmission was observed from health care workers to patients [35-38] highlighting an avoidable source of nosocomial infections.

It is frequently discussed where exactly *S. aureus* is colonising the human nose. On one hand, staphylococci were detected in the anterior region of the nose [39, 40] which consists of moist, stratified, squamous epithelium. The fact that this region is lacking cilia and therefrom mediated mechanical clearance is regarded as beneficial for the settlement of bacteria [40]. On the other hand, it was shown that *S. aureus* is able to colonise the rear nasal cavity with pseudostratified, columnar, ciliated epithelium [41, 42]. *S. aureus* are not just attaching to the host cells via the

expressed adhesins (reviewed in [43]); it was shown that this bacterium survives intracellularly in the nasal epidermis of the anterior nares [44].

Regarding the immunological status, intermittent and non-carriers are much more alike than persistent *S. aureus* carriers as excellently reviewed by Mulcahy and McLoughlin [45]. Persistent carriers exhibit elevated levels of antibodies against superantigens of their very own strain [46]. At the same time, non-carriers and intermittent carriers show similar eradication mechanisms [13]. The well-established mutual adaptation of host and coloniser is corroborated by the re-selection of previous persistent *S. aureus* strain when the host is inoculated with a mixture of different strains [13]. Moreover, the mortality of infections caused by colonising staphylococci is lower than infections of non-colonising strains [11]. Based on the host immune system and response, several genetic variations were shown to be predisposing for colonisation [47-51].

Nevertheless, staphylococcal colonisation poses an elevated risk for invasive and life-threatening infections [11, 52, 53] and therefore tries to be eradicated. The first choice for *S. aureus* nasal de-colonisation is mupirocin [54], which is applied on the nasal mucosa topically [55]. The protein synthase inhibitor [56] induces an effective staphylococcal clearance for a few weeks. However, a re-colonisation was observed within a remarkably high proportion of people [57, 58]. Surprisingly, more than a third of the people were re-colonised with the same strain which was identified in the first place [59]. The low effectivity of several antibiotics, including mupirocin, against intracellular staphylococci could explain the poor outcome of long-term de-colonisation [60].

1.2 The incidental pathogen *S. aureus*

In most cases the relationship between *S. aureus* and humans is of commensal nature [61]. However, under specific circumstances *S. aureus* can turn from commensal to pathogen by settling to other body sites and causing infections there. These pathogenic interactions can be classified by time (acute vs. chronic), location (bone, bloodstream, skin, lungs, etc.), and severity (e.g. superficial vs. life threatening). *S. aureus* is the most common contaminant of surgical wounds with the majority originating from endogenous flora [62]. This phenomenon demonstrates the importance of considering hitherto unnoticed staphylococcal colonisers as the source of nosocomial infections. In general, the disruption of the skin barrier or mucosal integrity by puncturing [26] (dialysis, catheter), surgery [62], burns [63], chronic wounds [64], or chronic disease related impairment (atopic dermatitis [65], cystic fibrosis [66]) was shown to be a major factor in the colonisation of the respective sites. Nevertheless, the development of life threatening infections based on colonisation is only observed in the minority of cases, even in patients with the mentioned predisposing factors [67].

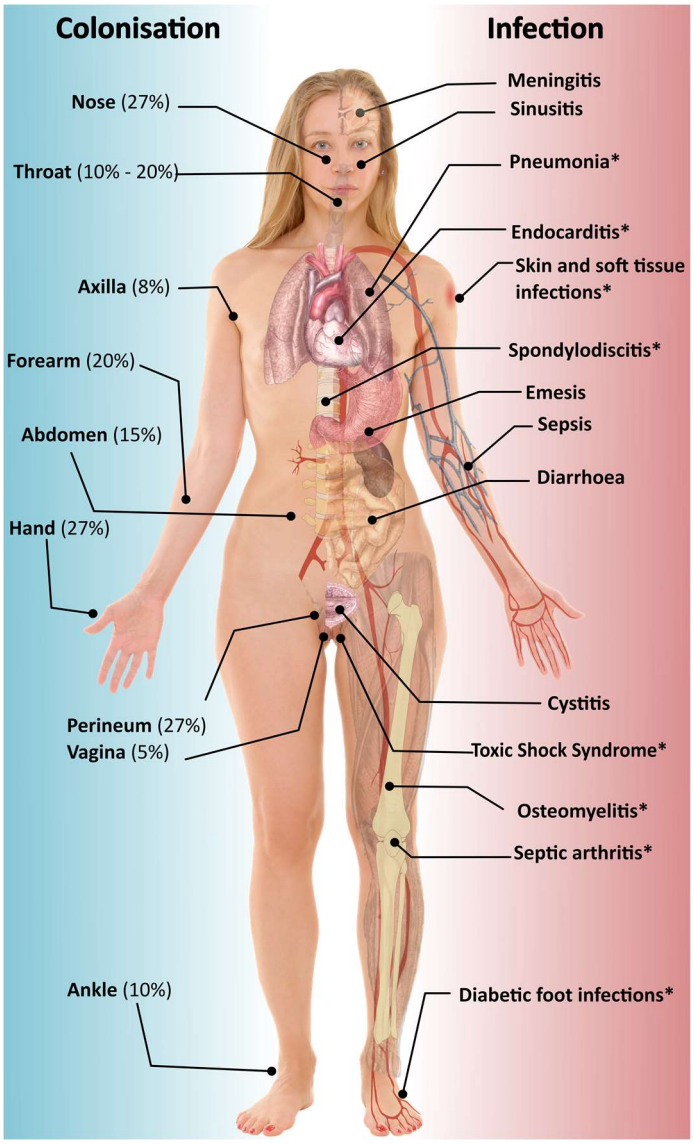


Figure 2 Diversity of staphylococcal colonisation niches and pathogenic interactions. * *S. aureus* is the main causative pathogen for the mentioned infection. References of indicated data are listed in Table S 1.

The knowledge about the mechanism of transition from healthy to pathogenic lifestyle in *S. aureus* is very poor. Nevertheless, a high age, diabetes mellitus, obesity, and vascular diseases are known predisposing factors on the host side [68]. Jenkins et al. simulated the actual process of transition by a three-phase cotton rat infection model [69]. It could be shown that the adhesins *sdrC* and *fnbA*, the metal acquisition genes *fhuD* and *sstD,* and the toxin *hla* were upregulated for the development of an invasive disease from nasal colonisation. Nevertheless, it should be critically noted that the mentioned study observed, with 23 genes analysed, only a limited amount of the actual gene expression. It is possible that crucial genetic regulations were overlooked due to the limitations of the study.

However, the development of an infection is equally determined by host and pathogen. With the help of mathematical modelling, a double-switch motif was identified for *Streptococcus pneumoniae*, an opportunistic pathogen which can cause invasive lung infections [70]. The discovered motif includes the "R-switch" which represents the activation of toll-like receptors (TLRs) by the bacteria. The "S-switch" is the growth of transmigrated bacteria in the blood stream over a certain threshold. The combination of the state of those switches resulted in different pathologic and non-pathologic situations. The investigation of the existence of comparable central motifs in *S. aureus* should be investigated when appropriate data are available to train the model. It is likely that TLRs play a similar important role in infections with *S. aureus* as naturally occurring SNPs in the genes which code for TLRs and have been shown to change the susceptibility for infections with the named pathogen [71]. Furthermore, it is known that host "danger signals" (especially febrile temperature), which are triggered by a viral lung infection, promote the staphylococcal dispersal from colonisation sites in the nose to future infection sites in the lung [72]. The reduction of microflora diversity was shown to be beneficial for *S. aureus* overgrowth [73, 74] and therefore facilitate the transition to a more pathogenic bacterial lifestyle.

1.2.1 The course of an invasive infection

Even though the transition to the pathogenic lifestyle occurs relatively rarely, the absolute numbers for staphylococcal infections are high due to the ubiquitous presence in and on humans. An infection is defined as invasive when bacteria are isolated from naturally sterile body sites or fluid, like blood, cerebrospinal fluid, or joint fluid [75, 76]. Staphylococci can access these locations by direct introduction into deeper structures in context of an operation or insertion of foreign devices in the human body. It is also possible that staphylococci spread from primary infection foci to other body sites via the blood stream. *S. aureus'* extensive arsenal of adhesins and virulence factors enables the bacterial survival in the blood stream by ensuring nutrient supply and fighting and evading the human immune system.

1.3 "Go with the flow" – bacteraemia

1.3.1 Staphylococcal strategies to overcome host nutritional immunity

When staphylococci are present in the blood stream, they are facing two major threats: the nutritional limitation and the potential bactericidal human immune response. In case of *S. aureus,* nutritional immunity is mainly mediated by the reduction of free available iron to a level which does not allow extracellular microbiological growth [77]. However, *S. aureus* developed strategies to circumvent this iron shortage (s. Figure 3). The main iron source is haem, complexed in the protein haemoglobin which is deliberated from erythrocytes by secreted bacterial haemolysins. The free haemoglobin is ingested via the iron-regulated surface determinant (Isd) system [78].

Moreover, *S. aureus* expresses the two siderophores staphyloferrin A and B which are competing with host iron complexing proteins transferrin and lactoferrin [78].

1.3.2 Interaction and manipulation of the immune system

Besides the restriction of nutrients, the human host fights actively against bacteria. Components of *S. aureus'* counterattack are summarised in Figure 3. Thereby, the innate immune response acts first. Neutrophils account for 60% of the leukocytes in the human blood and have a crucial role in the eradication of bacterial pathogens [79]. *S. aureus* secretes factors (SSL5, SSL10, CHIPS) which obstruct the chemotactic-based migration of neutrophils to the site of infection in the tissue [80 , 81]. However, if staphylococci are phagocytosed by neutrophils, they face several deadly weapons like antimicrobial peptides (AMPs), reactive oxygen (ROS), and nitrogen species [82, 83]. Staphylococci are equipped for those situations with the pigment staphyloxanthin which increases the resistance against ROS [84] and flavohemoglobin which detoxifies nitric oxide (NO) [85]. Moreover, the positive-charged bacterial cell wall, aureolysin, and staphylokinase (SAK) dispose AMPs [86-88]. Additionally, *S. aureus* developed a more aggressive strategy to survive upon phagocytosis by lysing phagocytes from within by expression of the pore-forming toxins phenol soluble modulin – α (PSMα) and α-toxin (Hla) [89-91]. If bacteria are outside of neutrophils, the latter can excrete neutrophil extracellular traps (NETs). The mixture of released neutrophil DNA, histones, AMPs, and proteases traps pathogens [92, 93]. Again, *S. aureus* comes up with a defence strategy in the shape of nucleases which digest the NETs [94].

Apart from the direct tackling of neutrophils, *S. aureus* prevents its own opsonisation by manipulating the complement system. Therefore, the bacteria secrete aureolysin which cleaves the central complement regulator C3 and inhibits the C3 convertase with the staphylococcal complement inhibitor (SCIN) resulting in a diminished opsonisation [95, 96]. The opsonisation of bacteria is essential for the recognition by neutrophils as dangerous; therefore, if this process is disabled, the whole immune response is attenuated [97].

The originally tightly regulated human coagulation system is hijacked by *S. aureus* as well. The possession of the factors staphylocoagulase (CoA) and the von Willebrand factor binding protein (vWbp) are species defining characteristics. Both factors can directly activate the coagulation cascade by binding to prothrombin and inducing a conformational change to staphylothrombin which now can convert fibrinogen to fibrin [98]. The generated fibrin is subsequently complexed with a large amount of aggregated bacteria creating a solid clot [99]. The binding between the individual staphylococci and between staphylococci and fibrin is mediated by the bacterial surface bound clumping factor A (ClfA) [100]. Even though in some situations the trapping of staphylococci in fibrin clots is beneficial for the host [101, 102], in blood stream infections, the

advantage is on the bacterial side [103]. The solid clot of staphylococci interwoven with host fibrin creates on one hand a shelter from antibiotics [104, 105], and on the other hand, an aggregate which is too big to be swallowed by phagocytes [106]. Moreover, the "activation" of prothrombin by staphylococci is not based on proteolytic cleavage, which restricts the enzymatic activity on fibrinogen. Therefore, the physiological thrombin-mediated activation of proinflammatory signals and antibacterial agents, like bradykinin, is prevented [97].

On the other side, *S. aureus* is armed with many leukotoxins which provoke the generation of proinflammatory signals by active destruction of immune cells. The toxins Panton-Valentin leukocidin (PVL) and leukocidin AB/GH (LukAB/GH) can lyse white blood cells by creating pores in their outer membrane [107]. The same mechanism is found for leukocidin ED (LukED) and γ-toxin (HlgACB) [108-111].

There are indications that erythrocytes play a special role for the bacterial clearance in the blood flow [112, 113]. The researchers propose, that due to the high velocity, leukocytes are unable to phagocytose and pathogens can only be bound and destroyed by red blood cells [114].

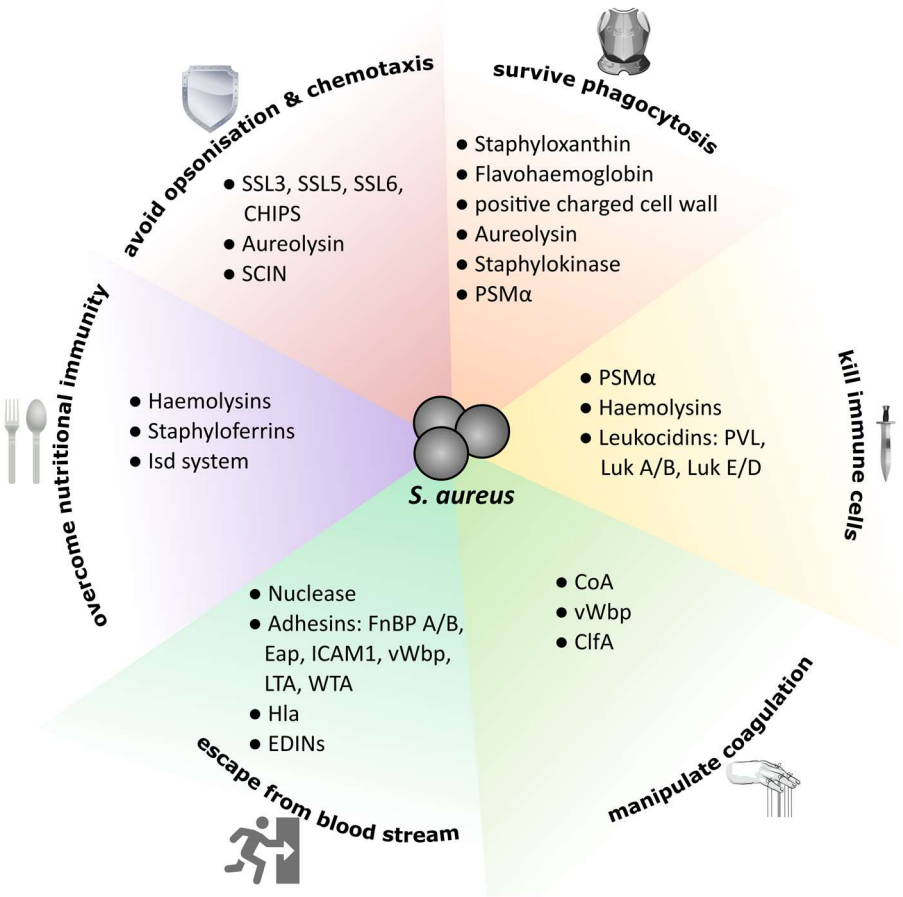


Figure 3 Key components for *S. aureus*' survival in the bloodstream and escape to deeper tissue structures.

1.3.3 Adhesion to vascular endothelium

As *S. aureus* is facing the constant threat of a potentially deadly environment in the vasculature, strategies to leave the blood stream evolved in this pathogen. The retention time of staphylococci in the blood stream is very short as they are not detected in the blood 24 h post infection (p.i.) [115, 116]. The first step of the escape is the adherence to the endothelial tissue by single bacteria or agglutinated bacteria-fibrin clots [115]. This process is mediated by several adhesins which are categorised by their linkage to the bacterial surface into microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) and secretable expanded repertoire adhesive molecules (SERAMs) [117]. In this context the most prominent representatives of the first adhesin family are the fibronectin-binding proteins A and B (FnBPA/B) which bind fibronectin on the surface of endothelial cells [115, 118]. The role of FnBPs is especially delicate as they trigger the bacterial uptake into host cells by bridging between fibronectin and integrin [119, 120]. This could represent a transcytotic way of leaving the bloodstream. The extracellular adherence protein (Eap) from the SERAM family can bind the endothelial cell adhesion molecule (ICAM-1) and can create an attachment by re-binding to the bacterium [121]. The previously described vWbp can bind to its eponymous von Willebrand factor which in turn binds to the endothelium [99]. There are also empiric evidence for the influence of the non-proteinaceous adhesin lipoteichoic acid (LTA) and wall teichoic acid (WTA) on the attachment to endothelium [122, 123].

1.3.4 Escape from the blood stream

Once the bacteria are attached to the interior surface of the blood vessels, various ways of blood stream escape are thinkable. One option is FnBP-mediated transcytosis through endothelial cells as mentioned above where the way of exit on the basal side is not definitely resolved yet [106]. If pathogens are able to disrupt the intercellular connection between host cells, they can pass over the paracellular route [124]. *S. aureus* probably accomplishes this with the help of α-toxin which, binding to the host receptor A Disintegrin and Metalloprotease 10 (ADAM10), activates acid sphingomyelinase leading to the destruction of tight junctions [125, 126]. By using professional phagocytes as a "Trojan horse", *S. aureus* turns a disadvantageous situation to its own advantage. The bacteria can survive within a vacuole in the phagocytic cells and reach distant body sites without causing the attention of the immune system [127-130]. After a few days, the cellular shelter is lysed by bacterial toxins and bacteria are set free [129]. An additional way of conquering the endothelial barrier is based on the attachment of a bacteria-interstratified thrombus. The formation of the thrombus is triggered by the pro-inflammatory host response after the FnBPs' mediated bacterial invasion into the endothelial cells [131]. The unleashed inflammatory reaction together with bacterial toxins damage the endothelial tissue so that deeper structures become

exposed [131]. In contrast to that, the formation of transient macroapertures represents a less damaging way of translocation. These tunnels stretch from the luminal to the basal site of the cell and are induced by the manipulation of the host actin cytoskeleton by bacterial epidermal cell differentiation inhibitors (EDINs) [132, 133].

1.4 Sepsis

The described battle between host and pathogen does not occur without major side effects for the infected person. In the worst case, the staphylococcal bacteraemia develops into sepsis which is defined presently as "life-threatening organ dysfunction caused by a dysregulated host response to infection" [134]. With 35 million yearly cases of sepsis worldwide and estimated 5.3 million deaths, *S. aureus* is the second leading pathogen causing this life threatening syndrome [8, 135-137]. As a result of the high incidence with its concurrent severity and expensive treatment costs [138], fast and exact diagnosis and treatment of *S. aureus* sepsis is strongly required.

1.4.1 Sepsis diagnosis

The fast, correct diagnosis of sepsis and the causing pathogen is of immense importance. It is suggested to save a blood sample before the onset of antimicrobial therapy in order to use the sample for the identification of circulating pathogens [139]. However, the timespan until reliable identification of the causing pathogen is 6 h to 5 d [140], highlighting the conflict between the need for correct information and the obligation of a fast therapy start. The gold standard for identification of pathogens in the blood is still blood culture with subsequent resistance testing. Indeed, there are several promising nucleic acid amplification technologies which unfortunately are limited regarding the range of detected pathogens, resistance spectrum, required amount of blood or requirement of elaborated sample preparation and handling [141].

1.4.2 Sepsis pathogenesis

Literally the bare existence of staphylococci in the bloodstream provokes pro-inflammatory host reactions as the bacterial cell wall components are recognised as pathogen-associated molecular patterns (PAMPSs) by host pattern recognition receptors (PRRs) [142]. Lipoprotein, peptidoglycan, and lipoteichoic acid induce the secretion of tumour necrosis factor α (TNF-α), interleukin 6 (IL-6), IL-1, and interferon-γ (IF-γ) by mononuclear phagocytes [143-145]. Additionally released bacterial DNA is quickly recognised as PAMP by the immune system [146]. However, some *S. aureus* strains are equipped with superantigens which crosslink major histocompatibility complex (MHC) class II with the T-cell receptor [147]. This link results in the unregulated activation of T-cells and their subsequent proliferation [148]. As described above, all strains possess pore-forming toxins (PSMs and Hla), whereas some strains are additionally

equipped with toxins that specifically harm leukocytes (PVL, LukAB/GH, LukED and Hlg). The destruction of white blood cells and host tissue generates damage-associated molecular patterns (DAMPs) which act as a second trigger for the progression of sepsis [142]. By causing an unphysiological, necrotic cell death, formerly intracellular compounds leak out and are recognised by the immune system as danger signal [142].

In sepsis, the overall failure of homeostasis is regarded as equally important as the immunological derailment [149]. Even though *S. aureus*-evolved strategies inhibit physiological coagulation in connection with the generation of unphysiological staphylothrombin, natural blood clotting is not prevented. As PAMPs are activating inflammatory mediators, the expression of tissue factor (TF) on primarily monocytes is promoted, too [150, 151]. In turn, TF is mainly responsible for massive thrombin generation in sepsis, leading to coagulopathy [150]. The generated platelet-fibrin clots block the blood flow and increase the risk of bleeding as they consume excessive amounts of anticoagulant factors at the site of coagulation [152, 153]. The pathogen-induced NETs act as additional coagulation activators [154, 155]. As the coagulation system and the complement system are interconnected, coagulation activates the complement system. Therefore *S. aureus* attempts to prevent opsonisation via secretion of SCIN [156]. Together the TF-mediated coagulation, dysfunction of anticoagulation, and activation of complement, form a deadly triad which is known as disseminated vascular coagulopathy (DIC) [151]. The occurrence of DIC is linked to a poor prognosis [157]. The smallest blood vessels, known as the microvascular system, directly provide the tissue. In these vessels, the bacterial disturbance has the highest impact. In these structures the blood flow is slower than it is in larger vessels, which facilitates the bacterial adhesion to the endothelium [158, 159]. Moreover, the generation of thrombi leads to a total blockage of these vessels and subsequent undersupply of associated tissue. Thrombus formation acts together with other factors, like metabolic disentailments of the mesenchymal tissue, which causes organ damage and finally failure [158]. In addition to the induced coagulopathy, the bacterial expression of toxins for blood stream escape and the generally inflamed endothelium contributes to the increased endothelial permeability and resulting undersupply of tissue [160-164].

1.4.3 Sepsis therapy

It was shown that the fast initiation of antimicrobial therapy within a few hours after the occurrence of the first symptoms with the right antibiotic is decisive for the survival of the patient [139, 165, 166]. The given insight of the interaction of *S. aureus* with the host represents only a small aspect of the multiple action and reaction in the fight for survival. Nevertheless, these examples demonstrate the high level of co-evolution and mutual adaptation in the arms race of infection. At the same time, the known interactions state many putative therapy targets and the

risk that accompanies the manipulation of them. This contradiction has led to the failure of more than 100 clinical studies on sepsis treatment [167].

The treatment of sepsis usually starts with antimicrobial therapy. Therefore, it is important that the therapy starts quickly (within 1h after diagnosis) with an antibiotic agent inside the bacterial susceptibility range. As described above, the diagnosis of the sepsis causing pathogen requires more than the requested 60 min to date. For this reason, a broad spectrum antibiotic is administered initially and replaced by a more specific antibiotic as fast as possible [168]. In case of an *S. aureus*-induced sepsis, β-lactam antibiotics should be chosen for methicillin-susceptible *S. aureus* (MSSA) [169]. For MRSA no uniform recommendation exists so far [169]. Furthermore, intravenous fluid should be administered to counteract tissue hypoperfusion.

As coagulopathy is a fatal characteristic of sepsis, several therapeutic approaches tackle that symptom. The amount of physiological anti-coagulants reduces in sepsis which is the reason anti-coagulants are administered [170]. However, if anti-coagulants are given in the later stages of sepsis, it is rather detrimental, as it can increase the risk of internal bleeding [151, 171]. Other therapy approaches that directly interfere with the human immune system by removing inflammatory mediators or addition of immune stimulators do not contribute to an improvement for all sepsis patients [172]. This finding indicates that the therapy must be adapted to the immune status of the patient, the stage of the infection, and the pathogen. Altogether, these challenges highlight the necessity of theranostics and personalised therapy [172]. One approach in that direction could be the division of patients into four different endotypes depending on representative markers [173]. As the development and manifestation of sepsis are very diverse, an individual assessment of the holistic clinical picture is of outstanding importance. Moreover, it was shown that the consultation of infectious disease experts for therapy advise, drastically lowers the mortality [169].

1.5 “To the bone” - osteomyelitis

Starting from the described bacteraemia or sepsis, *S. aureus* is able to settle at new infection foci in the body. Amongst others, the skeletal apparatus, especially the bone, is prone to staphylococcal infections. Osseous tissue represents a very intricate system as it is constantly renewing and remodelling itself. The main actors in this process are osteoblasts, which generate bone, and osteoclasts, which resorb bone. The unmineralised scaffold of the bone matrix is produced by osteoblasts and consists in the large part of collagen I fibrils. The remaining share is composed of proteins, e.g. fibronectin, osteopontin, and bone sialoprotein [174]. At the point where an osteoblast is completely enclosed by the self-produced matrix, it differentiates from an osteocyte

[175]. The mineralisation is also regulated by osteoblasts via the membrane bound enzyme alkaline phosphatase (ALPL) [176].

Bone infections are mostly caused by *S. aureus* [177, 178]. Osteomyelitis can be divided into categories depending on the origin of the pathogen. In case of haematogenous osteomyelitis, the pathogen settles from another infection in the body to the bone. Alternatively, bacteria can be introduced directly to the bone e.g. in context with an orthopaedic prosthesis implantation [174]. Due to the better perfusion of developing bones in children, haematogenous osteomyelitis accounts for 80% of bone infections in paediatric patients [179]. In contrast, most prosthetic implant operations are conducted on elderly patients for which reason this is the main source of bone infections [180].

Although "osteomyelitis" denominates strictly speaking the infection of the bone marrow, cortex, and periosteum, adjacent soft tissue is affected, too [178]. Initially triggered by the infecting microorganism, leukocytes produce pro-inflammatory cytokines and enzymes that degrade the osseous tissue [181]. As a consequence of the fight between host immune system and pathogen, an abscess is formed (Figure 4). As the abscess separates bone material from vascularised structures, like the periosteum, enlargement of the abscess leads to the interruption of blood supply. In these chronic infections, the blood perfusion impairments create devitalised and necrotic bone material, named sequestrum [182]. Cells of the immune system as well as systemic antibiotics cannot access the infection site due to the disconnection from the blood flow [174]. As the generation of sequestra is often attended by a loss of stability, a new bone, an involucrum, is generated to cope with the pressure of the carried weight [180]. Ultimately, a sinus tract can be formed which is a tunnel between the abscess and the skin surface [178]. In the case of vertebral osteomyelitis, up to 50% of all patients show neurological deficits as the abscess puts ressures on the spinal cord and nerves [183-185].

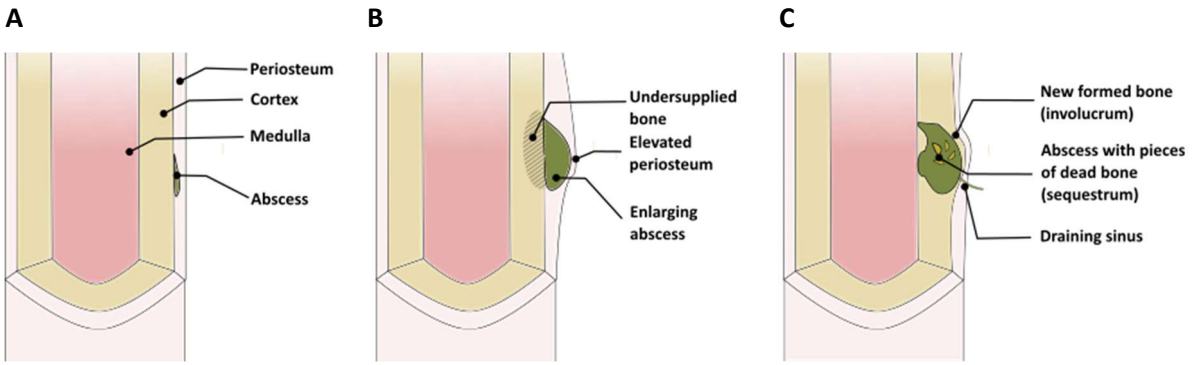


Figure 4 Development of osteomyelitis. A An abscess develops between the vascularised periosteum and the hard bone (cortex). **B** The enlargement of the abscess causes the lift of the periosteum, which results in the blockage of blood supply to the hard bone. **C** The undersupplied regions of the bone become necrotic, together with the abscess, the sequestrum is formed. If the periosteum is massively elevated, new bone formation occurs (involucrum) around the abscess region creating an encasing shell. Ultimately a sinus tract can spread from the abscess and devitalised structures, through soft tissue and skin. Illustration based on Kavanagh et al. [174] and McNally & Nagarajah [186].

The infection can develop from an initially easier treatable acute infection to a hard-to-cure chronic infection [178, 187]. Therefore, the time frame for the diagnosis should be as small as possible in favour of the therapeutic success. All bones in the human body can be infected with *S. aureus* where the spine is usually accessed via the bloodstream [188, 189].

1.5.1 Osteomyelitis diagnosis

Many of the initial symptoms of spondylodiscitis (spine infections) and osteomyelitis are rather unspecific and multiform. Therefore, the diagnosis needs to include the various aspects of these infectious diseases in order to create a significant picture. Osteomyelitis can be diagnosed with the help of a score established by Schmidt et al. [190]. The score includes data on the anamnesis, clinical parameters with special emphasis on inflammatory markers, imaging diagnostics, microbiology, and histology. The diagnostic procedure for spondylodiscitis is in principle similar [191].

1.5.2 Osteomyelitis pathogenesis

The first step towards infection is adhesion to the bone cells, plasma proteins, or extracellular matrix. This process is crucially mediated by staphylococcal MSCRAMMs. Similar to their action in the blood flow, FnBPs can bind plasma proteins, resulting in a connection to the host cell receptors [192, 193]. Moreover, the collagen adhesin (Cna) and the bone sialoprotein binding protein (Bbp) are of special importance as Cna is the only staphylococcal factor that binds collagen and Bbp mediates attachment to bone sialoprotein and fibrinogen [194-197]. Additionally, the SERAM extracellular adherence protein (Eap / Map) binds bone sialoprotein, fibronectin,

fibrinogen, and osteopontin [100, 198]. Although the surface of orthopaedic implants is initially abiotic, it is covered with extracellular matrix (ECM) components and immune proteins within a split second [199, 200]. This process creates an organic layer which enables bacterial adhesion in an identical way as described for native bone material. Additionally, *S. aureus* can attach to uncoated, abiotic surfaces by means of unspecific forces [201, 202].

Besides attachment, *S. aureus* interacts with the bone material by secretion of toxins. Many of these toxins were described previously for their action within in the blood stream. The α-toxin acts in a similar way on all cell types by inducing pore formation in the cell membrane and therefore causing cell death [203]. A comparable mechanism is observed for PSMs which was shown to have a cytotoxic effect on osteoblasts [204-207]. Another familiar player is CoA which induces apoptosis and simultaneously decreases osteoblast proliferation and mineralisation [208]. The species-defining protein A (Spa) and the relatively rare Toxic shock syndrome toxin 1 (TSST-1) enhance both bone resorption by osteoclast activation [209-211]. Altogether, the listed toxins contribute to the shift towards bone cell reduction.

As already described for endothelial cells, the binding of FnBPs to integrin on host cells can trigger internalisation of *S. aureus* [212]. The uptake of bacteria by osteoblasts usually leads either to apoptosis due to the activation of intracellular tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) or to bacterial persistence in the intracellular compartment [213, 214]. The exact process of persistence will be elaborated later.

Conversely, *S. aureus* can also survive outside of bone cells by establishing a biofilm. This lifestyle is of special importance for infection of orthopaedic implants [215]. After changing from planktonic to the sessile mode, staphylococci form microcolonies and generate a protective biofilm [216]. The second phase is characterised by bacterial propagation and establishment of a functional biofilm matrix including water channels for nutrient and waste exchange [217]. Latest findings indicate a very heterogeneous nature of biofilms as their composition varies at different stages and on different locations within the same biofilm [218]. Due to the increasing cell density, the staphylococcal quorum sensing system activates the accessory gene regulator (Agr) which in turn induces the expression of PSMs. These factors are responsible for the generation of water channels and in later stages for the biofilm dispersal in the last stage of the cycle [217]. The life within a biofilm poses several advantages which are similar to the intracellular shelter. The biofilm protects the bacteria from antibiotics, the immune system, and shear stresses. At the same time the accumulation of staphylococci in direct proximity allows the genetic exchange via conjugation and creates a heterogenous population with well-adapted subpopulations for a changing environment [219].

The presence of *S. aureus* in the usually sterile bone triggers an immunological reaction of osteoblasts. The bacterial PAMPs activate PRRs extracellularly (mostly toll-like receptors TLRs, TNF-α receptor 1) and intracellularly nucleotide-binding oligomerization domain (NOD) and NOD-like receptor [220]. As a reaction upon PRR stimulation, the cells secrete chemokines (e.g. regulated on activation, normal T cell expressed and secreted = RANTES) that attract osteal macrophages and neutrophils [220]. Additionally, the attraction of Th1 lymphocytes as part of the adaptive immunity was observed [221]. The summoned macrophages are supposedly the main producer of the pro-inflammatory mediators IL1, IL-6, and TNF-α [220]. Exactly those three cytokines are known for their promoting effect on osteoclast function and differentiation resulting in bone resorption [222]. The inflammatory response in implant infections is even more pronounced as the implant is recognised as foreign material by the immune system. The following extensive immunological reaction is conversely beneficial for the pathogen as the abiding release of ROS induces a metabolic absorption of the neutrophils [223-225].

1.5.3 Osteomyelitis therapy

A general treatment guideline for osteomyelitis in adults does not exist at the moment [174, 191]. Ideally the infecting pathogen should be identified from a bone biopsy [226]. The isolated bacterium should be tested for antimicrobial susceptibility, which would define possible treatment options. Suitable antimicrobial agents are extensively reviewed by Kavanagh et al. [174]. For a long time, an intravenous antibiotic therapy of 4 – 6 weeks was an unquestioned standard in osteomyelitis therapy [227]. However nowadays this administration is under debate due to missing advantages in comparison with oral application [187, 228, 229]. This changing dogma can be attributed to the availability of antibiotics with high oral bioavailability and good bone penetration [174, 230]. Complementary, local antibiotic delivery systems are used to reach a high antimicrobial concentration at the site of infection [231, 232].

The surgical debridement is inevitable in chronic osteomyelitis after all [178]. By removing the necrotic sequestrum, a viable vascularised environment is generated which potentially favours bone recovery and hinders bacterial replication and spread [178, 233]. In spondylodiscitis, surgical therapy is only used if the abscess is causing neurological symptoms and / or pain [191]. In contrast to that, prosthesis infections usually always acquire surgical intervention as the necrotic tissue and orthopaedic implant need to be removed in order to eradicate the attached microbiological biofilm [216, 234, 235]. After surgery, the resulting empty space is preferentially filled with autologous bone grafts [236, 237].

Recurrent infections are a major complication in osteomyelitis as up to 40% of the patients are affected. Those relapses can ultimately lead to the exigency of limp amputation [174].

1.6 Intracellular persistence

As highlighted before, *S. aureus*' ability to persist inside different cell types poses a major threat for human health [238]. Staphylococci developed several mechanisms to deal with the imprisonment in phagosomes of professional and non-professional cells.

Compared with professional phagocytes, some non-professional phagocytes develop extenuated endosomes which are characterised by a low pH and antibacterial activity [239, 240]. If *S. aureus*-containing phagosomes mature unaffectedly or if the maturation is detained, it seems to be dependent on host cell type and / or staphylococcal strain [240]. Indeed, *S. aureus* was shown to be able to inhibit the fusion of phagosome and lysosome and therefore prevents its lysosomal degradation [241]. However, if phagosomal acidification is successful, *S. aureus* can be able to resist in this kind of environment or de-acidify it [240, 242].

Another coping strategy is based on the escape from host cell phagosomes. The phagosomal escape is mainly mediated through the expression of PSMs, especially PSMα [243, 244]. The PSM-induced pore formation in the endosomal membrane acquires additional factors (e.g. LukAB) to be effective [245]. Like many other virulence factors, *psm* expression is under the control of the *agr* regulon, which is why knock out of *agr* result in disabled phagosomal escape [245].

S. aureus adaptation is not only limited to plain survival in the intracellular compartment; it can even replicate inside host cells. The exact location for this process appears to be dependent on the type of cell as staphylococci replicate inside professional phagocyte's phagosomes; whereas in non-professional phagocytes, replication takes place in the cytosol or autophagosomes [240].

As a symptom of adaptation to the intracellular environment, staphylococci switch to the small colony variant (SCV) phenotype [246]. SCVs exhibit an approximately 10-times smaller colony size on an agar plate compared to wild type colonies [247]. Defects in the electron transport chain or thymidine synthesis lead to stable SCVs [238]. The SCV phenotype is characterised by a slowed growth, a decrease in respiration, pigmentation, haemolysis, coagulase activity, and a general unstable colony phenotype [248]. In contrast, the resistance towards some antibiotics is increased [249, 250]. Additionally, SCVs secrete more adhesins and fewer toxins [247, 251]. The combination of the listed characteristics leads to a perfect adaptation for the intracellular environment. The fact that the proportion of SCVs increases with time of intracellular cultivation, underlines this last statement [247]. This is consistent with the fact that SCVs are regularly detected in clinical specimen from chronic infections e.g. osteomyelitis patients [248]. Clinical

SCVs are usually not stable and revert quickly back to the wild type phenotype upon subcultivation [252]. The dynamic phenotype switching to the SCV phenotype is caused by the regulator sigma factor σB (SigB), which silences the *agr* regulon [253, 254]. The latter regulator is a major regulators of virulence factor expression and was shown to be important for the establishment of an acute infection *in vivo* and debilitating for bacterial persistence in chronic infection [254]. The tight regulation of the dynamic phenotype switch and its success in chronic infections, demonstrate the importance of SCV formation as a persistence mechanism inside host cells. Moreover, new infection outbreaks can rise from persistent bacteria. This connection explains the high *S. aureus* recurrence rates up to 33% [255]

1.7 Tailor-made changes? - of ecology, evolution and virulence

The high expression of a variety of virulence factors in the bloodstream and the dynamic formation of "silent" SCVs emphasises two antagonistic survival strategies within the same bacterium. The development of this pattern can be enlightened with regard to evolutionary processes.

Evolutionary success is defined by means of fitness which is in turn described as the successful production of offspring. According to this definition, species with a high fitness will increase over time in number and outgrow competitors with a lower fitness [256]. It is generally accepted that virulence is an unavoidable symptom of host exploitation in favour of the pathogen survival, reproduction, and transmission to a new host [257]. The existence of competing bacterial species will select for the higher virulence, resulting in disadvantages in the transmission and promoting negative selection [257]. Virulence is defined as the decrease of host fitness which can be translated in a medical microbiological context to "harm or morbidity to the host" [258]. The definition of a "pathogen" is more complex than it initially seems as it is not just a disease-causing agent. As Méthot and Alizon highlighted in their configurational model, a pathogen can only be identified and defined in relation to its host and environment [259]. This construct implies that commensalism and parasitism are constantly merging and the development of a disease is equally shaped by host and bacterium [259]. The fact that severe diseases like sepsis are mainly caused by the immunopathology of the host itself emphasises this last assumption [260].

1.7.1 Development of bifunctional toxins

Opportunistic pathogens, like *S. aureus,* exist in various environments and relations to their host. This variety can lead to a pre-adaptation in an asymptomatic compartment, e.g. in the nose, and a subsequent selection in infectious contexts [258]. The resulting virulence factors are multifunctional as observed in *S. aureus*. For example PSMs have been shown to have a toxic effect on *Streptococcus pyogenes* [261], a common coloniser of the human respiratory tract [262]. Several membrane damaging toxins have important roles in the establishment, construction, maintenance, and dispersal of bacterial biofilms [263-265]. Moreover, toxins like PSMs and α-toxins exhibit very effective defensive functions by enabling bacteria to escape from intracellular phagosomes [266]. Another function of the versatile PSMs is the function as a surfactant-like substance which adds mobility to a technical immobile bacterium [267]. Even the superantigens, which are probably the most fatal toxins, were found to have a rather harmonious side as they regulate the staphylococcal density during nasal colonisation thereby preventing bacterial overgrowth and elimination by the immune system [268].

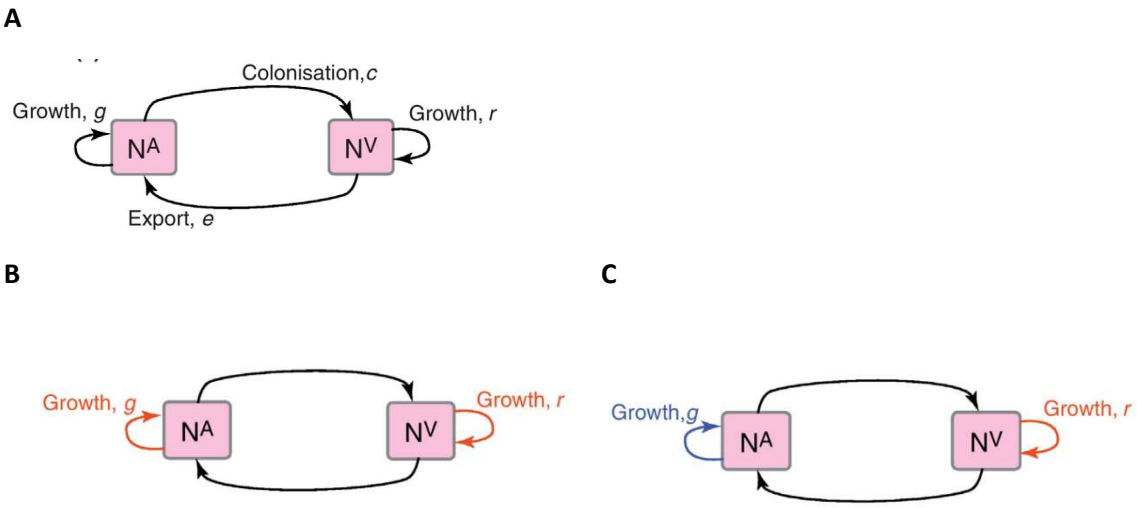


Figure 5 Ecological and evolutionary dynamics that influence the development of virulence factors. **A** General model. NA and NV represent bacterial densities in the asymptomatic (A) and virulence sites (V), respectively. Arrows represent demographic processes of growth (g, r) and transmission (colonisation c, export e). **B** Model for pre-adaptation. If properties in the asymptomatic and virulence sites are positively correlated, pre-adaptation can occur. Positive selection in either environment optimises the population for survival in the other environment. **C** Model of environmental trade-off. If the benign and the virulent environment are negatively correlated, adaptation to one environment is attended by a disadvantage in the other environment (trade off). Red arrows: positive selective impact, blue arrows: negative selective impact. Figure and text modified from Brown et al. 2012 [258]

However, not all staphylococcal factors are beneficial for both habitats. In such a case the expression of those factors would be linked to a disadvantage or environmental trade-off [258]. Indeed, the trade-off can be avoided by the dynamic change of gene expression. The prerequisite of this phenotypic plasticity is the sensing of the environment. *S. aureus* is equipped with an elaborated assortment of monitoring systems to modulate virulence and antibiotic resistance depending on environmental changes [269-271].

1.7.2 Quorum sensing induced regulation of cytotoxicity in *S. aureus*

The *agr* quorum sensing system is central to the adaptive regulation of cytotoxicity in *S. aureus*. The *agr* locus codes for two divergent transcripts [272]. In the upstream direction RNAII includes *agrB, agrD, agrC, agrA*. In the opposite direction the transcript RNAIII acts as small regulatory RNA and contains the gene for haemolysin delta at the same time. The RNAII-associated promoter P2 is permanently active on a low level. The transcribed Agr proteins have very distinct functions in the generation, perception, and reaction onto quorum sensing signals. AgrD represents a precursor of the autoinducing peptide (AIP). This precursor is transported extracellularly and maturated by the *agrB*-coded multifunctional transmembrane protein.

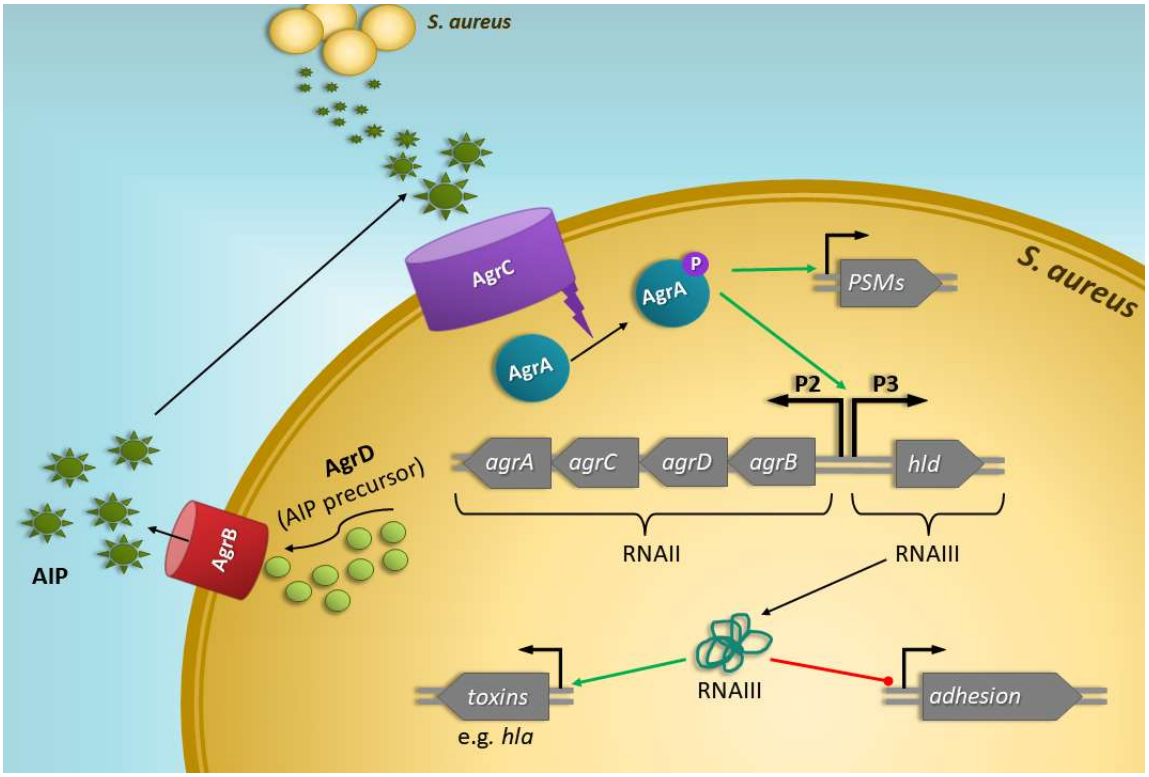


Figure 6 Quorum sensing induced regulation of cytotoxicity in *S. aureus*. High bacterial densities lead to accumulation of extracellular AIP, which activates AgrC. The cytosolic kinase of activated AgrC phosphorylates AgrA that acts as a transcription factor for RNAII and RNAIII. The RNAII sequence includes genes for AgrB, AgrD, AgrC, and AgrA. The transcribed RNAIII acts mainly as small regulatory RNA which promotes the transcription of toxins and inhibits the transcription of genes for adhesion and biofilm formation. The mRNA for haemolysin delta is contained in RNAIII. AIP = autoinducing peptide.

If staphylococci accumulate locally and the level of AIP rises upon a certain threshold, the membrane histidine kinase ArgC is activated by autophosphorylation. This in turn results in phosphorylation of intracellular AgrA, the central response regulator of this system. The activated

AgrA binds to the *agr* locus and induces the activation of the afore mentioned promoter P2 and the divergent promoter P3. The latter initiates the transcription of RNAIII which is an important post transcriptional regulator as it influences the expression of a multitude of virulence factors (haemolysin α/β, enterotoxins, leukocidins, exoproteases) positively and the expression of cell surface proteins (protein A, permease, coagulase, oligopeptides, adhesins) negatively. Moreover, AgrA directly activates the transcription of several targets, e.g. *psmαβ* [273]. With the help of this system, *S. aureus* regulates its cytotoxicity in relation to bacterial density which enables dispersion from mature biofilms and dissemination within the body. Recently it was shown that the quorum sensing machinery is sensible to NO which inhibits AgrA [274]. NO is usually generated by macrophages to kill intracellular bacteria [275]. *S. aureus*' susceptibility to this host defence mechanism was reviewed as a way to enable virulence as well as commensalism [274]. This underlines once again the importance of both lifestyles for the bacterium.

1.8 Objectives

The previous introduction illustrated the versatility and flexibility of *S. aureus*, especially the pathogenic side of this bacterium, emphasising the exigency of research in this field. Better understanding of the staphylococcal "behaviour", with its adaptation mechanisms, lays the foundation for an improved treatment and cure of *S. aureus* infections.

Therefore, this present study attempted to clarify the significance of a fixed bacterial genotype and / or phenotype for the induction of a certain disease. Moreover, a comprehensive picture of every strain was assembled with the aid of various techniques.

This study had the following objectives:

1. To establish a *S. aureus* strain collection from three distinct pathologies and nasal colonisation. Strains were selected by several patients' characteristics in conjunction with a matching detection pattern of *S. aureus* to ensure a reliable group assignment. Further patient characteristics were assessed in parallel.
2. To analyse the genetic background and gene expression of central regulators and virulence genes. The genotyping was realized by using a DNA microarray; whereas, the gene expression analysis was determined by quantitative real-time PCR.
3. To characterise the phenotypes for infection properties in basic models and cell culture models. This has been done by testing the strains for their haemolysis ability on sheep blood erythrocytes. Moreover, invasiveness, cytotoxicity, and potential for immunological stimulation were assessed in osteoblast infection assays where results were retrieved by fluorescence-activated cell scanning (FACS) and ELISA.
4. To determine the persistence ability in cell culture and animal models. Selected strains were used for the long-term persistence model in osteoblasts. The number of intracellular bacteria was determined on several time points, and wild type colonies and SCVs were also distinguished. Moreover, strains were tested in murine sepsis model.
5. To analyse correlations to identify distinct phenotypic patterns. In all results, group-dependent and independent correlations were investigated using bioinformatic tools.

The results of the present dissertation were published in Tuchscherr, L.; Pöllath, C.; Siegmund, A.; Deinhardt-Emmer, S.; Hoerr, V.; Svensson, C.-M.; Thilo Figge, M.; Monecke, S.; Löffler, B. Clinical *S. aureus* Isolates Vary in Their Virulence to Promote Adaptation to the Host. *Toxins* 2019, *11*, 135. The data were re-evaluated and interpreted for this work.

2. Material and methods

2.1 Material and instruments

Table 1 Instruments

Instrument	Characteristic	Company (town, country)
Bacteria incubator	Heraeus B12	Heraeus (Hanau, Germany)
Cameras	Canon PowerShot G16, Canon EOS 1100D	Canon (Tokyo, Japan)
Cell counter	TC 20™	Bio-Rad (Hercules, CA, U.S.A.)
Cell culture freezing container	Mr. Frosty™	Thermo Fisher Scientific™ (Waltham, MA, USA)
Cell incubator	LAB.INC.A/J T/C CO2 W/DCN	Thermo Fisher Scientific™ (Waltham, MA, USA)
Centrifuges	5810 R, 5417 R	Eppendorf (Hamburg, Germany)
Clean bench	Biowizard golden line	Kojair (Vilppula, Finnland)
Colony counter	colonyQuant	Schuett Biotec (Göttingen, Germany)
Electrophoresis power supply	PowerPac 200 Power Supply	Bio-Rad (Hercules, CA, U.S.A.)
Electroporation device	Gene Pulser II + Controller	Bio-Rad (Hercules, CA, U.S.A.)
enzyme-linked immunosorbent assay (ELISA) reader	Infinite 200Pro	Tecan (Männedorf, Switzerland)
FACS	BD Accuri C6+	BD Biosciences (Erembodegem, Belgium)
Gel documentation station	Molecular Imager ® GelDoc XR System	Bio-Rad (Hercules, CA, U.S.A.)
Homogeniser	FastPrep-24	MP biomedicals (Shandong, China)
Inverse light microscope	Nikon TMS	Nikon (Tokyo, Japan)
Magnetic stirrer	RH basic	IKA (Staufen, Germany)
Photometer	Ultrospec 2000 UV/VIS	Pharmacia Biotech (Uppsala, Schweden)
Pipetting robot	QIAgility	Qiagen (Hilden, Germany)
Quantitative Real time PCR cycler	Rotor-Gene Q	Qiagen (Hilden, Germany)
Shaking incubator	TH 30	Edmund Bühler GmbH (Tübingen, Germany)
Sonication bath	SONOREX SUPER RK 100 H	Bandelin (Berlin, Germany)

Spektrophotometer	NanoDrop ND-1000 Spectrophotometer	Peqlab, Erlangen, Germany
Thermocycler	Biometra® TRIO- Thermoblock TM + TRIO Heated Lid	Biometra biomedizinische Analytik GmbH (Göttingen, Germany)
Tissue homogeniser	Polytron® 2500	Kinematica (Luzern, Switzerland)
Vacuum pump	BVC professional	Vaccubrand (Wertheim, Germany)
Vortex mixer	WIZARD IR Infrared Vortex Mixer	VELP Scientifica (Usmate Italy)
Water bath	GFL 1003	GFL (Großburgwedel, Germany)

Table 2 Chemicals and Kits

Chemical	**company**
Agarose	Serva (Heidelberg, Germany)
cDNA synthesis QuantiNova Reverse transcription Kit	Qiagen (Hilden, Germany)
Chloroform	Carl Roth (Karlsruhe, Germany)
Dimethyl sulfoxide	Sigma-Aldrich (St. Louis, MO, U.S.A)
Ethanol	VWR International (Radnor, PA, U.S.A)
Ethidiumbromide	Carl Roth (Karlsruhe, Germany)
Isopropanol	Carl Roth (Karlsruhe, Germany)
Nuclease-free water	Thermo Fisher Scientific™ (Waltham, MA, USA)
Plasmid Midi Kit	Qiagen (Hilden, Germany)
Propidium iodide	Sigma-Aldrich (St. Louis, MO, U.S.A)
QIAquick PCR Purification Kit	Qiagen (Hilden, Germany)
Sodium bicarbonate ($NaHCO_3$)	Carl Roth (Karlsruhe, Germany)
Sodium carbonate (Na_2CO_3)	Sigma-Aldrich (St. Louis, MO, U.S.A)
TRIzol™	Thermo Fisher Scientific™ (Waltham, MA, USA)

Table 3 Media (cell culture and bacteria culture)

Medium	**company**
Accutase Solution	PromoCell (Heidelberg, Germany)
brain heart infusion (BHI)	Oxoid (Hampshire, U.K.)
Collagenase	Sigma-Aldrich (St. Louis, MO, U.S.A)
Dexamathasone	Sigma-Aldrich (St. Louis, MO, U.S.A)

Endothelial Cell Growth Medium with supplement	PromoCell (Heidelberg, Germany)
FBS superior	Biochrom (Berlin, Germany)
Fibronectin	Roche (Basel, Switzerland)
Ham's F12 DMEM/F12, no phenol red	Thermo Fisher Scientific™ (Waltham, MA, USA)
HAT Supplement (50X)	Thermo Fisher Scientific™ (Waltham, MA, USA)
Human serum albumin (HSA) Albunorm	Octapharma (Lachen, Switzerland)
Hydroxyethyl-piperazineethane-sulfonic acid buffer (HEPES-buffer)	Biochrom (Berlin, Germany)
L-Ascorbic acid 2-phosphate sesquimagnesium salt hydrate	Sigma-Aldrich (St. Louis, MO, U.S.A)
Medium 199	Lonza (Basel, Switzerland)
Mueller Hinton	Oxoid (Hampshire, U.K.)
Minimum essential medium α (α-MEM) with nucleotides	Biochrom (Berlin, Germany)
β-Glycerophosphate disodium salt hydrate, BioUltra	Sigma-Aldrich (St. Louis, MO, U.S.A)

Table 4 Antibiotics

Product	**Company**
Geneticin® Selective Antibiotic (G418 Sulfate)	Thermo Fisher Scientific™ (Waltham, MA, USA)
Gentamycin	Ratiopharm (Ulm, Germany)
Lysostaphin	Ambi Products LLC (Lawrence, NY, U.S.A.)
Penicillin / Streptomycin	Biochrom (Berlin, Germany)

Table 5 Oligonucleotides

Gene name	**Primer description**	**Primer sequence**	**Product length**	**Reference**
agrA	Forward	5'-AACTGCACATACACGCTTACA-3'	145 nt	[247]
	Reverse	5'-GGCAATGAGTCTGTGAGATTT-3'		
gyrB	Forward	5'-AATTGAAGCAGGCTATGTGT-3'	138 nt	[247]
	Reverse	5'-ATAGACCATTTTGGTGTTGG-3'		
hla	Forward	5'-CAACTGATAAAAAAGTAGGCTGGAAAGTGAT-3'	201 nt	[276]
	Reverse	5'-CTGGTGAAAACCCTGAAGATAATAGAG-3'		
psmα	Forward	5'-GCCATTCACATGGAATTCGT-3'	151 nt	This institute
	Reverse	5'-CAATAGCCATCGTTTTGTCCT-3'		
rnaIII	Forward	5'-TTCACTGTGTCGATAATCCA-3'	70 nt	[251]
	Reverse	5'-TGATTTCAATGGCACAAGAT-3'		
emp	Forward	5'-TGTTGCTAAAGGCCCAGAAG-3'	87 nt	This institute

	Reverse	5'-TTCTTGTAGTGGGTTTGCGTAG-3'		

Table 6 Consumables

Product	Company
12-well plates	Greiner Bio-One (Kremsmünster, Austria)
96-well F-bottom plates	Greiner Bio-One (Kremsmünster, Austria)
96-well V bottom plates	Greiner Bio-One (Kremsmünster, Austria)
Cell culture flasks (25cm^2, 75cm^2, 175cm^2)	Greiner Bio-One (Kremsmünster, Austria)
Cell scraper	Sarstedt (Nümbrecht, Germany)
Cuvettes	Sarstedt (Nümbrecht, Germany)
Pipette tips	Nerbe plus (Winsen, Germany)
Polystyrene round bottom tubes	Sarstedt (Nümbrecht, Germany)

Table 7 Software

Software	Company
BD Accuri™ C6 Software	BD Biosciences (Erembodegem, Belgium)
GraphPad Prism 6	GraphPad Software (San Diego, CA, U.S.A.)
MS Office	Microsoft Corporation (Redmond, USA)
PCR software, Rotor-Gene Q	Qiagen (Hilden, Germany)
SPSS Statistics 24	IBM (Armonk, NY, U.S.A.)

2.2 Methods

2.2.1 Study group and bacterial isolates

The bacterial isolates were obtained from a strain collection at the Jena University Hospital as well as from the microbiological routine diagnostic department at the Institute of Medical Microbiology. The sampling of strains and the documentation of selected patient data was approved by the local ethics committee of the University Hospital Jena (registration no.: 4874-07/16). The corresponding patients and healthy people resided in Thuringia and the adjacent federal states (Saxony-Anhalt, Bavaria, Hesse). No informed consent was needed as all testing was performed with clinical strains and not with patient samples. The isolates were grouped depending on the donor into the categories: sepsis, haematogenous osteomyelitis, prosthesis infection, and nasal isolates from healthy people.

2.2.2 Clinical definitions

Sepsis was defined according to the definition by the Third Sepsis Consensus Conference in the year 2016 as "[a] life-threatening organ dysfunction due to a dysregulated host response to infection." This is evaluated by the Sepsis-related Organ Failure Assessment (SOFA) score or the simplified form, the quick SOFA (qSOFA) [277]. The qSOFA criteria are fast respiratory rate, altered mental state, and lowered blood pressure. If two of the three criteria are fulfilled, the chances for a severe organ dysfunction, and therefore a sepsis, are high. The qSOFA was used in this work to identify patients for the sepsis group. Moreover, there are two groups with bone infections; whereas, the group of haematogenous osteomyelitis is defined by an additional *S. aureus*-positive blood culture. In turn, a history of bone operations with an implementation of a prosthesis and a negative blood culture define the prosthesis infection group. Group assignment was kindly done by Dr. Stefanie Deinhardt-Emmer.

2.2.3 Genotypic characterisation and statistical analysis

The genotype of the isolates was determined with the Alere StaphyType DNA microarray (Alere Technologies GmbH, Jena, Germany). Before the addition of the isolate DNA to the array, the DNA is amplified. In this process biotinylated desoxyuridine triphosphate (dUTP) is integrated into the product. The biotinylated product is transferred to an area with spotted complement DNA fragments of selected genes. Afterwards streptavidin is added which binds to the biotin of the initial product. The hybridisation is made visible by a subsequent colorimetric precipitation based on a streptavidin horseradish peroxidase reaction. The hybridisation pattern is detected and evaluated by an ArrayMate Reader. The array was performed by the company Alere. This method enables the assignment to clonal complexes (CCs) and the detection of 170 genes and their allelic

variants. The differences between the isolate groups and the CCs were analysed by two-sided Fisher's exact test with IBM SPSS Statistics 24.

2.2.4 Growth curve and generation time

For growth curves, *S. aureus* strains were grown at 37 °C with shaking (160 rpm) overnight in 5 ml of BHI. On the following day, 200 µl of BHI in a 96-well F-bottom plate were inoculated with each strain to obtain an optical density (OD) 0.05 (578 nm). The growth of each strain was monitored spectrophotometrically every 15 min over 16 h with a plate reader. The growth rate (µ, growth speed) and the generation time (g) for each strain used in this study were calculated according the standard formula by Madigan et al. [278].

2.2.5 Haemolysis assay

To assess the ability of the strains to secrete haemolytic toxins, a haemolysis assay was conducted. Therefore, defibrinated sheep blood was washed with Dulbecco's phosphate-buffered saline (DPBS) (500 xg 10 min) several times until the supernatant was clear from free haemoglobin. Then, a 1.7% solution was prepared in DPBS with the pelleted erythrocytes.

A previously described protocol was modified in the following way: 5 ml of a BHI medium in a conical flask were inoculated with a single colony of *S. aureus* patient isolates and incubated at 37 °C with an agitation of 160 rpm for 15 h. On the next day, the overnight culture was centrifuged at 12,000 rpm at 4 °C for 5 min to obtain whole culture supernatant. Then, 100 µl of the supernatant were mixed with the same amount of the previously prepared erythrocyte solution in a V-Bottom plate. The mixture was incubated at 37 °C, 5% CO_2 for 30 min. To separate intact erythrocytes from the supernatant with the released haemoglobin, the plate was centrifuged at 1000 rpm and 4 °C for 5 min. The supernatant was subsequently transferred to a new F-bottom plate. The free haemoglobin was quantified by absorbance measurement at 570 nm, whereas supernatant of the high haemolytic *S. aureus* strain Wood46 was used as positive control.

2.2.6 Cell culture

2.2.6.1 Isolation of primary cells (pHOBs, HUVEC) and cultivation of osteoblast cell line (hFOB 1.19)

Primary human osteoblasts (pHOBs) were kindly provided by Christian Fritzsch who isolated the cells according to the protocol by Dillon et al. [279]. The pHOBs were cultivated in α-MEM with addition of 10% FBS, penicillin / streptomycin (100 U/ml, 100 µg/ml), 0,2 mM L-ascorbic acid 2-phosphate, 10 mM β-glycerophosphate and 10 nM dexamethason.

For the isolation of human umbilical vein endothelial cells (HUVECs) umbilical cords were received from the Sophien/Hufeland-Klinikum, Weimar. The isolation was conducted under sterile conditions. At the beginning, the outside of the umbilical cords was manually cleaned with prewarmed DPBS. Then, the ends of the cord were cut with a scissor, and stainless steel luer adaptors were inserted into the larger vein. The luer adaptors were fixed with cable ties. A syringe with pre-warmed DPBS was connected to one luer adaptor and the DPBS was pushed through the vein until the emergent liquid was clear. Afterwards a syringe with collagenase (0.2% wt/vol in DPBS) was plugged to the other luer and the enzyme solution was pushed into the vein. The filled umbilical cord was incubated at 37 °C for 30 min. Then both syringes were removed, and the vein slowly flushed with pre-warmed Medium 199. During this process, the cord was manually massaged to detach as much cells as possible. All of the liquid was collected in a 30 ml falcon tube. The obtained cell suspension was centrifuged 5 min 1.000 xg and the supernatant was removed subsequently. The remaining cell pellet was resuspended in HUVEC full medium (Endothelial Cell Growth Medium with supplement + 10% FBS + 100 U/ml, 100 µg/ml penicillin / streptomycin) and seeded into one well of a 6-well plate which was incubated at 37 °C. After the cells reached confluence, they were detached with 500 µl accutase-solution and seeded into a fibronectin coated, 75 cm^2 cell culture bottle. For coating 1 ml of pure fibronectin (1 mg/ml in ddH_2O) was pipetted and suspended in a 75 cm^2 cell culture bottle.

The osteoblast cell line hFOB1.19 was grown at 34 °C where these cells exhibit a short doubling time and the phenotype of an immature osteoblast. The basic medium Ham's F12 DMEM/F12 contained additionally 10% FBS and 0.3 mg/ml G418. G418 is an aminoglycoside antibiotic which is used to select for a desired, genetically modified cell type. As it is toxic for wild type eukaryotic cells and bacteria, this additive was omitted for infection experiments.

2.2.6.2 Maintenance of cell culture in general

Cells were routinely stored in the liquid nitrogen phase at −196 °C. If needed, cells were thawed as quick as possible in a 37 °C water bath. The thawed cell suspension was subsequently mixed with 9 ml pre-warmed cell culture medium matching to the specific cell type. In order to remove the residual freezing medium, the cell suspension was centrifuged at 500 xg for 5 min. After removing the supernatant, the cell pellet was resuspended in 4 ml and 11 ml of fresh medium respectively and seeded into 25 cm^2 or 75 cm^2 cell culture flasks. The cells were cultivated at a humid atmosphere with 5% CO_2 at 34 °C for hFOB1.19 and 37 °C for pHOB and HUVEC. The medium was changed every second day and the cell culture was subcultivated when it reached at least 80% confluence. In detail the cells were washed once with room temperature DPBS followed by the addition of Trypsin / EDTA (for hFOB1.19, pHOB) or accutase (for HUVECs). After 3-5 min at the standard incubation temperature, the detachment of cells was controlled microscopically.

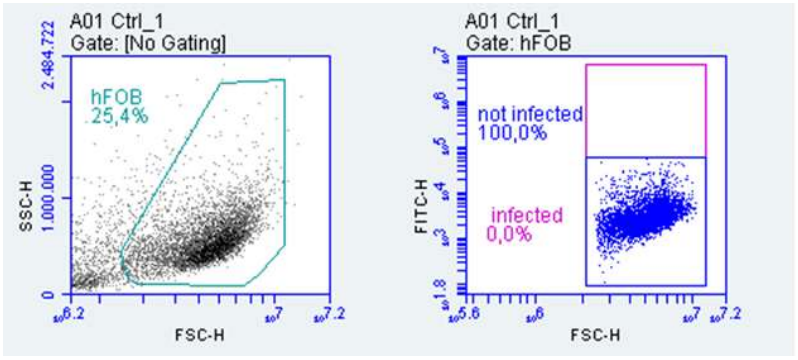
Detached cells were resuspended in an adequate amount of full medium and seeded into a cell culture plate or flask. When cells needed to be frozen for long time storage, cells were washed and detached as described for subcultivation. The detached cells were counted with a cell counter. After a 5 min centrifugation at 500 xg, the cell pellet was resuspended in a respective amount of freezing medium (basic medium + 8% DMSO) to adjust the cell concentration to 0.5 – 1x10^6. The cell suspension was aliquoted into cryogenic vials which were transferred to a pre-cooled freezing container. This container prevented the formation of big ice crystals by slow cooling. The filled container was stored for 15 min at 4 °C and for 24 h at -80 °C before the vials were moved to the liquid nitrogen tank.

2.2.7 Invasion assay

An invasion assay was used to determine the invasiveness of the *S. aureus* isolates and mutants. The cells were seeded two days prior the infection into 12-well plates to obtain a confluence of 80% - 90% on the day of infection. The cells were washed with 0.5 ml DPBS followed by the addition of 0.5ml invasion medium (basic medium + 25mM Hepes+ 1% HSA).

Based on the protocol of Tuchscherr et al. [280], bacteria were prepared. First, an overnight culture of 5 ml of Mueller-Hinton Broth was inoculated with a single bacterial colony and incubated at 37°C. The overnight culture was centrifuged at 4 °C and 4,000 rpm for 5 min the next day to pellet the bacteria. The pellet was washed with DPBS and sonicated for 1 min before it was fixed with 3% formaldehyde in ddH_2O for 2 h at room temperature. Following a washing step with DPBS, the bacteria were stained with fluorescein isothiocyanate (FITC) for 1 h at 37°C. Specifically FITC (0.1 mg/ml FITC in DMSO) was mixed with a buffer containing 1.55% $NaHCO_3$ and 3.35% Na_2CO_3 to obtain the final staining solution. After the incubation time passed, the bacteria were washed with DPBS and their OD at a wavelength of 578 nm (OD^{578nm}) was adjusted to 1 in DPBS. For the infection, 100 µl/well of the defined bacterial solution were incubated with the host cells for 1 h at the standard cultivation temperature. The infection process was stopped by removing the medium with the bacteria and washing the host cells with DPBS. To detach and singularise the infected host cells, all wells were treated with 200 µl/well trypsin / EDTA solution (0.05% / 0.02%). After 5 min of incubation at 37 °C, 5% CO_2, the reaction was stopped by adding 500 µl/well DPBS + 10% FBS. The mixture was gently pipetted up and down several times and finally transferred to individual round bottom polystyrene tubes. The tube was centrifuged at a speed of 180 xg for 5 min at 4 °C. The supernatant was removed, and the pellet was resuspended in 500 µl of DPBS. The internalized bacteria were quantified by the measurement of the fluorescence of FITC with a flow cytometer as described previously [281].

A



B

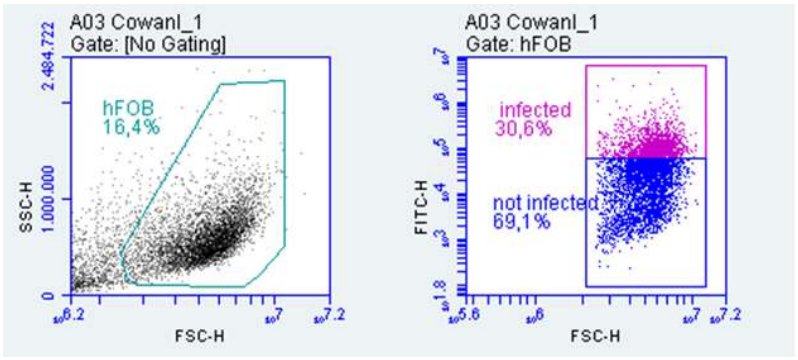


Figure 7 Exemplary representation of FACS gates for determination of invasiveness in osteoblasts (hFOBs). A Gates were adjusted to uninfected host cells to correct for autofluorescence of measured osteoblasts. **B** The high infecting strain *S. aureus* CowanI was used as positive control.

2.2.8 Cell death assay

2.2.8.1 General protocol for the cell death assay

Host cells and bacterial overnight culture were prepared in the same way as described for the invasion assay. The overnight culture was washed with DPBS and subsequently adjusted to an OD of 1. To singularise the bacterial cells the bacterial suspension was sonicated for 1 min. Then, the host cells were infected with 50 µl/well of the bacterial suspension for 1.5 h. Following a washing step with DBPS, 1 ml of gentamycin medium (basic medium + 10% FCS + 200 µg/ml gentamycin) was added and incubated at the standard incubation temperature within an atmosphere with 5% CO_2 to kill all extracellular bacteria. After an incubation of 1 h cells were washed with DPBS and fresh full medium was added. The cells were incubated under standard conditions overnight. To collect all cells independently from their attachment to the plastic surface, all liquids from washing and detaching were pooled into one tube. The procedure was the same as described for the invasion assay. The addition of 50 µl propidium iodide of a 50 µg/ml working solution marked the dead cells so that the latter could be quantified by flow cytometry.

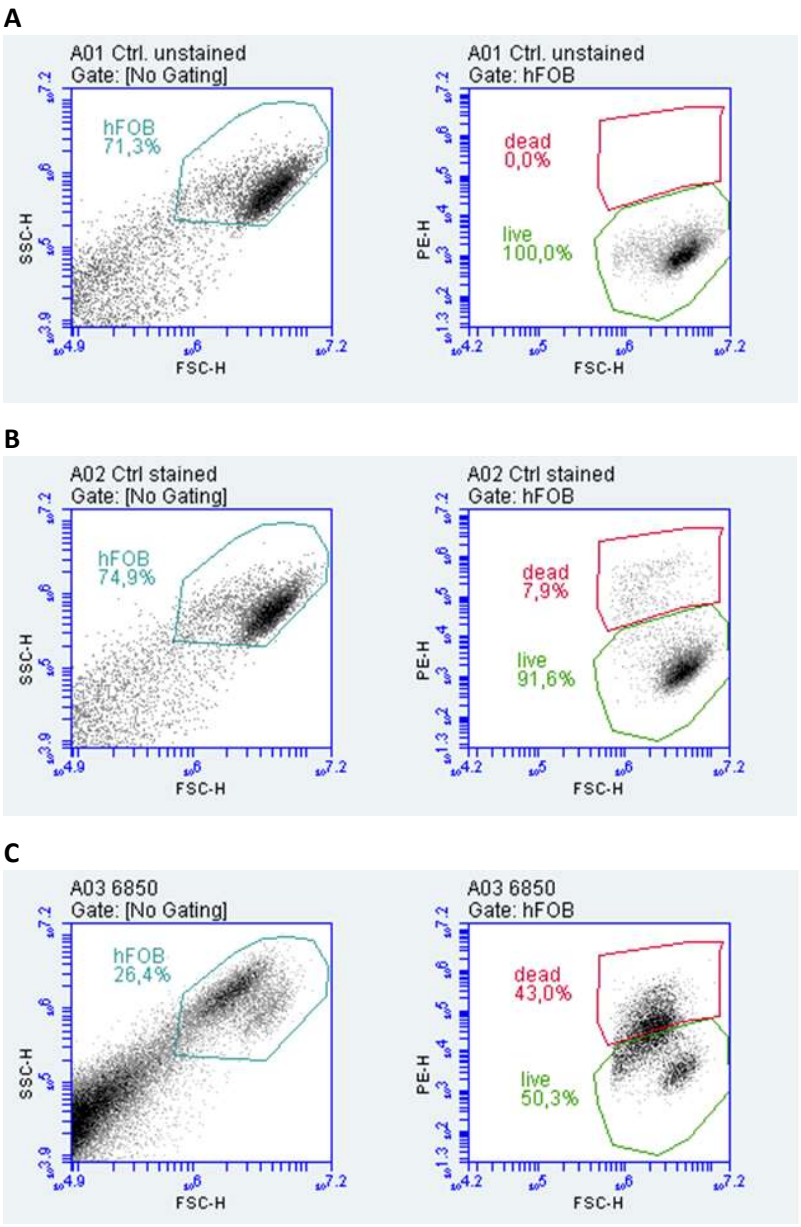


Figure 8 Exemplary representation of FACS gates for determination of cytotoxicity on osteoblasts (hFOBs). A Gates were adjusted to uninfected, unstained host cells to correct for autofluorescence of measured osteoblasts. **B** An experiment was only conducted if the cell death of uninfected cells was lower than 10%. **C** The high cytotoxic strain *S. aureus* 6850 was used as positive control.

2.2.8.2 Testing of different cell types for invasion and cell death assay

To ensure the observed differences between the strains were dependent on the specific host cell type, the assays were conducted with human cells of different backgrounds. Selected standard strains were used which are known for their high invasiveness (*S. aureus* CowanI), high cytotoxicity (*S. aureus* 6850), or low invasiveness (*S. carnosus* TM300) and low cytotoxicity (*S. aureus* CowanI). It was observed that the high invasive strain (*S. aureus* CowanI) invaded the host cells on a high level independently of the cell type. The strain *S. carnosus* TM300 invaded all

types of cells to a significantly lower extent, whereas the highest values were measured in the experiment with pHOBs as host cells. For the cytotoxicity assay the general mortality of the uninfected host cells was decisive for the number of dead cells after infection. The osteoblast cell line (hFOB 1.19) showed the lowest values and the primary endothelial cells (HUVECs) were most likely to die with or without infection. However, when high and low cytotoxic bacteria were compared, the pattern always remained the same. All in all, the specific type of host cells played only a minor role when different strains were compared as the relation between high and low value strains persisted independently of the host cell background. For the sake of time and effort, the osteoblast cell line hFOB 1.19 was used for the screening of the clinical isolates and the persistence assay.

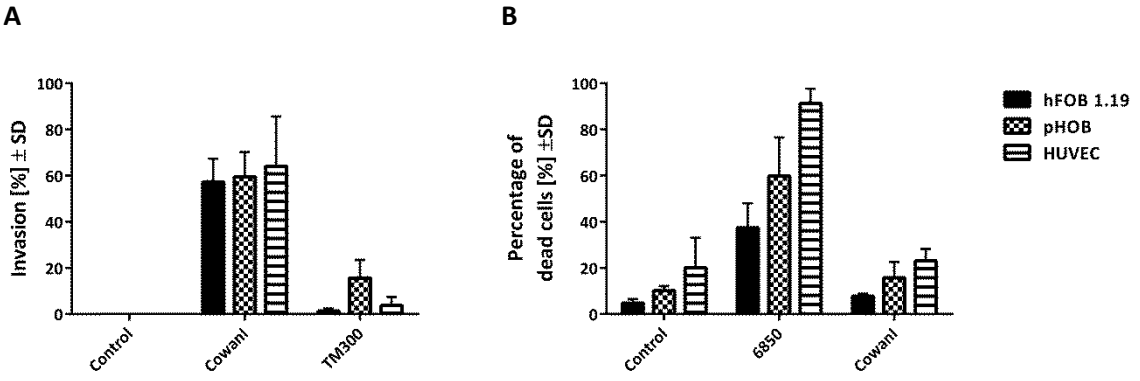


Figure 9 Comparison of osteoblast cell line (hFOB 1.19) with primary human osteoblasts (pHOB) and primary human umbilical vein endothelial cells (HUVECs) for **A** invasion and **B** cytotoxicity assay. **A** The high invasive strain invaded all types of cells to a similar degree. The low invasive strain (*S. carnosus* TM300) showed the highest invasion when they were co-cultivated with pHOBs. **B** The high cytotoxic strain (6850) destroyed the most cells; whereas, the low cytotoxic strain showed cytotoxicity levels which were slightly higher than the uninfected control. In general, the number of dead cells was the lowest when the osteoblast cell line was used. Significance was tested with paired, two-sided t-test. The differences between the cell line hFOB 1.19 and the primary cells were not statistically significant.

2.2.9 Long-term persistence

The human osteoblast cell line hFOB 1.19 was infected with different *S. aureus*-patient isolates as previously described [282]. In short, the osteoblasts were infected with a multiplicity of infection (MOI) of 50. After a 90 min incubation, the cells were washed with DPBS and lysostaphin (20 µg/ml) was added for 30 min to lyse extracellular bacteria. Then, the medium was replaced by fresh cell culture medium with penicillin / streptomycin (100 U/ml, 100 µg/ml). The addition of penicillin and streptomycin were expected to prevent bacterial overgrowth during the experiment. Moreover, the infected cells were incubated with lysostaphin medium every two days and always before lysis. To determine the quantity and quality of the intracellular Staphylococci at different time points, p.i., host cells were lysed with 10 ml ice cold ddH_20 for 10 min and detached by scraping. Cell lysates were serial diluted and plated on Columbia blood agar plates.

The plates were incubated for 72 h and every 24 h colony forming units (CFUs) and SCVs were counted with a colony counter (Schuett colonyQuant, Göttingen, Germany). SCVs were defined by a diameter of <0.8 mm.

2.2.10 Measurement of the released chemokine RANTES

Osteoblasts (hFOB1.19) were seeded into 12-well plates and infected with staphylococci in a similar order as described for the long-term persistence model. The supernatant was removed 24 h and 48 h after the infection and stored at -80 °C. To determine the exact number of host cells, osteoblasts were detached, as described for the invasion assay, and quantified flow-cytometrically. The obtained supernatants and sera from mice were analysed with RANTES human Instant ELISA™ and RANTES Mouse Instant ELISA™ respectively (ThermoFisher Scientific).

2.2.11 Harvesting of bacterial culture, RNA isolation, qPCR

To determine gene expression in early stationary phase, BHI medium was inoculated with a single colony and incubated overnight at 37 °C with 160 rpm agitation. On the following day the culture was diluted to an OD^{578nm} of 0.05 and incubated at 37 °C, with 160 rpm. At the defined timepoint of 4 h (*agrA*, *rnaIII*) and 6 h (*psmα*, *hla*) 1 ml of bacterial suspension was transferred to a microcentrifuge tube and bacteria were pelleted by centrifugation for 5 min at maximum speed. After discarding the supernatant, the pellet was resuspended in 750 µl of TRIzol™ reagent.

On the next day, the overnight culture was diluted to an OD^{578nm} of 0.05 and incubated at 37 °C with 160 rpm. After 6 h of incubation, 1 ml of bacterial suspension was transferred to a microcentrifuge tube and centrifuged for 5 min at maximum speed to pellet the bacteria. The supernatant was removed, and the pellet was resuspended in 750 µl of TRIzol™ reagent. Samples were stored until further processing at -20 °C. For isolation of RNA the samples were thawed and incubated for 5 min on ice. Then the mixture was transferred to a Lysing Matrix B tube and homogenized with a Fast Prep homogenizer. The tubes were centrifuged 2 min 12,000 rpm at 4 °C with subsequent transfer of the supernatant to a new tube where the homogenate was incubated for 5 min on ice. Next, 200 µl of chloroform was added and shortly mixed. After 3 min of incubation on ice, samples were centrifuged 15 min, 12,000xg at 4 °C. The RNA-containing phase was transferred to a new tube where the RNA was precipitated with 500 µl of isopropanol for at least 2 h at -20 °C. Then the precipitate was pelleted at 4 °C, 12,000xg for 10 min. The pellet was washed one time with 1 ml of 75% ethanol, dried and resuspended in RNase free water. The concentration and purity of RNA were determined spectrophotometrically with a NanoDrop. The genomic DNA

(gDNA) digest and the complementary DNA (cDNA) synthesis were done with the QuantiNova Reverse transcription Kit according to manufactures instruction. The resulting cDNA solution was used for quantitative real-time PCR with QuantiNova SYBR Green PCR Kit in a Rotor-Gene Q thermocycler. The reaction mixtures were incubated for 15 min at 95 °C followed by 40 cycles of 15 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C. The primers are listed in Table 5. The fold change was calculated by the *Pfaffl* equation [283]. The housekeeping gene *gyrB* was used as a reference gene.

2.2.12 Animal model

The murine sepsis model was performed by Dr. Verena Hörr and Yvonne Ozegowski as described by Horst et al. [284]. C57BL/6 10-week-old female mice were obtained from central laboratory animal facility of the Jena University Hospital. The animals were maintained according to institutional guidelines in individually ventilated cages and were given food and water ad libitum. Mice were inoculated with $1x10^6$ CFUs of *S. aureus* in 200 µl of DPBS via a lateral tail vein and sacrificed by CO_2 asphyxiation after 3 d (acute) and 6 weeks (chronic) p.i.. To enumerate the bacteria in the tibiae of infected mice, homogenates were prepared in DPBS and plated in decadic serial dilutions on blood agar.

The murine sepsis model infection model was conducted in accordance with the recommendation and guidelines of the German regulations of the Society for Laboratory Animal Science 22-2684-04-02-006/15 and 22-2684-04-02-046/16 (Thüringen, Jena).

3. Results

3.1 *S. aureus* patient isolates of different origin showed just slight genotypic differences

3.1.1 Clinical characteristics of patients differ in a group-specific manner

In order to compare strains from different pathologic backgrounds, a collection of 47 bacterial isolates was established. The group of nasal colonisation from healthy people and the group of prosthesis infection included 12 strains. Ten strains were collected from sepsis patients and 13 strains isolated from patients suffering from haematogenous osteomyelitis. Selected medical characteristics are listed in Table 8. The age was comparable between the three different patient groups; whereas, people from the group of nasal colonisation were marginally younger. The percentage of men was raised in the group of prosthesis infections compared to the other groups which showed a more balanced sex ratio. The highest mortality was found for the haematogenous osteomyelitis patients where more than one third died from the infection. In the sepsis group every fifth case turned out to be fatal. In contrast to that, none of the patients from the prosthesis infection group died from *S. aureus* infection.

Table 8 Summarised characteristics of healthy people and patients.

	Nasal colonisation	Prosthesis infection	Haematogenous osteomyelitis	Sepsis	p[a]
Total number	12	12	13	10	
median age [years]	49.08 (range 40-64)	66 (range 33-85)	75.23 (range 47-96)	62.7 (range 53-78)	**<0.001**
Male	6 (50,0%) [b]	9 (75.0%)	8 (61.5%)	5 (50,0%)	0.738
Case fatality	-	0	5 (38.5%)	2 (20.0%)	**0.042**
Underlying condition					
Immunosuppression	-	2 (16.7%)	3 (23.1%)	3 (30.0%)	0.759
Diabetes mellitus	-	2 (16.7%)	5 (38.5%)	7 (70.0%)	**0.043**
Malignant disease	-	0	2 (15.4%)	2 (20.0%)	0.841
Indwelling devices	-	7 (58.3%)	2 (15.4%)	1 (10.0%)	**0.025**
Renal disease	-	5 (41.7%)	12 (92.3%)	7 (70.0%)	**0.024**
Cardiovascular disease	-	9 (75.0%)	8 (61.5%)	9 (90.0%)	0.345
Liver disease	-	3 (25.0%)	1 (7.7%)	4 (40.0%)	0.160
Mean number of comorbidities / patients	-	2.42	2.58	3.3	0.1365
Origin of infection / primary focus					
Unknown	-	0	3 (23.0%)	0	0.096
Bone	-	12 (100%)	7 (53.8%)	0	**≤0.001**
Wound / skin	-	0	1 (7.7%)	2 (20.0%)	0.268
Intravenous catheter	-	0	2 (15.4%)	0	0.318
Lung	-	0	0	6 (60.0%)	**≤0.001**
Gastrointestinal tract	-	0	0	2 (20.0%)	0.076

Hospital stays during last 3 years (in relation to infection)					
None	-	3 (25%)	10 (76.9%)	9 (90.0%)	
1	-	0	2 (15.4%)	0	
≥2	-	9 (75%)	1 (7.7%)	1 (10.0%)	
Median length of hospital stay [days]	-	60 (range 14-1080) [c]	30 (range 9-180)	17,5 (range 2-360)	0.0825

[a] binary variables were tested with Fisher's exact test, non-binary parametric variables were tested with one-way analysis of variance (ANOVA), whereas non-parametric binary variables were tested with Kruskal-Wallis test

[b] one sample could not be assigned to sex

[c] no data available for one isolate from the group of prosthesis infection

Regarding the underlying conditions of the patients, diabetes mellitus was found more often in the severe diseases of haematogenous osteomyelitis and sepsis; while more patients with prosthesis infections were dependent on indwelling devices at the same time. When both bone infection groups were compared, almost all patients in the haematogenous group were suffering from an impaired renal function. In contrast, in the other group, only half of the patients showed the same condition. The bacterial infection mainly originated from the bone in both bone-infection groups. In contrast, the lung was the predominant source of infection in the sepsis patients. Interestingly, the median length of hospital stay was the highest for the prosthesis infection group and the lowest for the sepsis group, whereas the group of haematogenous osteomyelitis stood in between.

3.1.2 Affiliation of clinical isolates to population groups

The affiliation of the *S. aureus* isolates to CCs were determined by genotyping (s. Table 9). In total, 13 different clonal complexes were observed among all tested Staphylococci. The distribution of

Table 9 Frequencies of the clonal complexes within staphylococcal isolate groups.

Clonal complex (CC)	Nasal colonisation (n = 12)	Prosthesis infection (n = 12)	Haematogenous osteomyelitis (n = 13)	Sepsis (n = 10)	p[a]
CC1	0	0	7,7% (1)	10,0% (1)	0,578
CC5	16,6% (2)	25% (3)	0	10,0% (1)	0,271
CC6	8,3% (1)	8,3% (1)	7,7% (1)	0	1,000
CC7	0	33,3% (4)	0	0	**0,007**
CC8	0	16,6% (2)	15,4% (2)	10,0% (1)	0,620
CC15	8,3% (1)	0	15,4% (2)	10,0% (1)	0,717
CC22	0	0	30,8% (4)	30,0% (3)	**0,019**
CC25	8,3% (1)	0	7,7% (1)	0	1,000
CC30	16,6% (2)	8,3% (1)	0	0	0,382
CC45	25% (3)	0	7,7% (1)	30,0% (3)	0,116
CC101	0	0	7,7% (1)	0	1,000
CC121	0	8,3% (1)	0	0	0,723
CC398	16,6% (2)	0	0	0	0,164

[a] p values were calculated with Fisher's exact test

clonal complexes in the defined groups was very diverse as every group contained at least six different CCs.

None of the CCs from this sample was found in all isolate groups; CC5, CC6, CC8, CC15 and CC45 were found in at least three of the groups. The CC7 was found in a high percentage and exclusively in isolates from prosthesis infections. Furthermore, in the two isolates groups which originated from blood infections, haematogenous osteomyelitis and sepsis showed similarities in the distribution of CCs. CC22 had the highest frequency in both groups and was not found in any of the other groups.

3.2 Prevalence of selected virulence factors among *S. aureus* isolates

The complete microarray hybridisation data are provided as a supplementary file (s. Table S 2). The data are displayed in three different ways to facilitate the evaluation. The combination of genes and isolate groups displayed in Table 10 and Table 11 contains the information about the frequency of genes within the clonal complexes. For the sake of clarity, rare CCs of this study (CC1; CC25; CC101; CC121, CC398) are summarised in one group in Table 11.

3.2.1 Regulation

In all the groups the main *agr* was *agrI,* with a frequency of 60% -70%. The gene *agrII* was found in all the groups, too; while 15% - 25% of all strains harboured that gene. Only a small proportion allotted to *agrIII* and *agrIV*. On a clonal level it was observed that CC5 was associated with *agrII* and CC30 with *agrIII.* All residual CCs were majoritarian associated with *agrI*.

3.2.2 Resistance

The genes of the β-lactamase operon were significantly more often found in the group of sepsis where almost every isolate carried those genes. Generally, genes coding for resistance against erythromycin, methicillin and tetracycline were relatively rare, and the *vanA* gene, which enables resistance against vancomycin, was not found at all. In contrast, approximately half of all strains had potentially a resistance against Fosfomycin mediated by the *fosB*; whereas, the frequency of this gene was lower in the sepsis group. Additionally, *fosB* showed a CC-specific distribution with high rates in the CCs 5; 6; 15; 30. Furthermore, the endowment with *sdrM* was significantly less in the groups of blood infections.

Table 10 Frequencies of selected genes in the defined isolate groups.

Gene	Product	Nasal colonisation	Prosthesis infection	Haemato-genous osteomyelitis	Sepsis	p[a]
Regulation						
agrI	Accessory gene regulator allele I	58.3% (7)	66.7% (8)	69.2% (9)	70.0% (7)	0,950
agrII	Accessory gene regulator allele II	25.0% (3)	25.0% (3)	15.4% (2)	20.0% (2)	0.928
agrIII	Accessory gene regulator allele III	16.7% (2)	8.3% (1)	7.7% (1)	10.0% (1)	0.927
agrIV	Accessory gene regulator allele IV	0	0	7.7% (1)	0	1.000
Resistance						
bla	β-lactamase	58.3% (7)	58.3% (7)	46.2% (6)	90.0% (9)	0.184
ermA	Erythromycin / clindamycin resistance gene A	0	16.7% (2)	0	20.0% (2)	0.171
ermB	Erythromycin / clindamycin resistance gene B	0	0	0	0	-
ermc	Erythromycin / clindamycin resistance gene C	0	8.3% (1)	0	0	0.723
fosB	Metallothiol transferase	58.3% (7)	66.7% (8)	53.8% (7)	30.0% (3)	0.419
mecA	Alternate penicillin binding protein 2, defining MRSA	0	16.7% (2)	7.7% (1)	10.0% (1)	0.612
sdrM	Putative Transport-/ efflux protein	100% (12)	100% (12)	69.2% (9)	70.0% (7)	**0.029**
tetK	Tetracycline resistance gene K	0	0	7.7% (1)	10.0% (1)	0.578
tetM	Tetracycline resistance gene M	0	8.3% (1)	0	0	0.723
vanA	Vancomycin resistance gene	0	0	0	0	-
Enterotoxins						
sea	Staphylococcal enterotoxin A	16.7% (2)	16.7% (2)	15.4% (2)	0	0.597
seb	Staphylococcal enterotoxin B	8.3% (1)	0	15.4% (2)	0	0.603
sec, sel	Staphylococcal enterotoxin C+L	25.0% (3)	0	15.4% (2)	10.0% (1)	0.332
sed, sej, ser	Staphylococcal enterotoxin D+J+R	0	25.0% (3)	7.7% (1)	20.0% (2)	0.244
see	Staphylococcal enterotoxin E	0	0	0	0	-
egc-cluster	Staphylococcal enterotoxin G+I+M+N+O+U	66.7% (8)	33.3% (4)	53.8% (7)	70.0% (7)	0.289
seh	Staphylococcal enterotoxin H	8.3% (1)	0	7.7% (1)	10.0% (1)	0.885
sek, seq	Staphylococcal enterotoxin K+Q	0	8.3% (1)	7.7% (1)	0	1.000
tst1	Toxic shock syndrome toxin (TSST)-1	16.7% (2)	0	0	0	0.164
entP	Enterotoxin P	0[b]	41.7% (5) [b]	0	10.0% (1)	**0.003**
Haemolysins						

hla	α-toxin	100% (12)	100% (12)	100% (13)	100% (10)	-
hlb	β-toxin	75.0% (9)	100% (12)	84.6% (11)	80.0% (8)	0.381
hld	δ-toxin	100% (12)	100% (12)	100% (13)	100% (10)	-
Leucocidins						
lukF, hlgA	γ-toxin	100% (12)	100% (12)	100% (13)	100% (10)	-
lukF-PV	Panton-Valentin-leucotoxin	8.3% (1)	0	0	0	0.723
lukD, lukE	Leucocidin D, E component	41.7% (5) [c]	91.7% (11) [c]	61.5% (8)	40.0% (4)	**0.034**
Exfoliative toxins						
etA	Exfoliative toxin A	8.3% (1)	0	0	0	0.723
etB	Exfoliative toxin B	0	0	0	0	-
etD	Exfoliative toxin D	8.3% (1)	0	7.7% (1)	0	1.000
Enzymes						
aur	Aureolysin	100% (12)	100% (12)	100% (13)	100% (10)	-
chp	Chemotaxis inhibitory protein (CHIP)	66.7% (8)	25.0% (3)	61.5% (8)	80.0% (8)	0.056
splA, splB	Serine protease A, B	41.7% (5)[d]	91.7% (11)[d]	61.5% (8)	40.0% (4)[d]	**0.034**
splE	Serine protease E	41.7% (5)	66,7% (8)[e]	46.2% (6)	10% (1)[e]	0.065
sspA, sspB	Glutamyl endopeptidase	100% (12)	100% (12)	100% (13)	100% (10)	-
sak	Staphylokinase	66.7% (8)[f]	100% (12)[f]	84.6% (11)	80.0% (8)	0.173
scn	Staphylococcal complement inhibitor	91.7% (11)	100% (12)	100% (13)	100% (10)	0.723
Exopolysaccharides						
cap5	Capsular polysaccharide 5	41.7% (5)	41.7% (5)	46.2% (6)	50.0% (5)	1.000
cap8	Capsular polysaccharide 8	58.3% (7)	58.3% (7)	53.8% (7)	50.0% (5)	1.000
icaA, D, C	Polysaccharide intracellular adhesin	100% (12)	100% (12)	100% (13)	100% (10)	-
Adhesins						
bbp	Bone sialoprotein-binding protein	91.7% (11)	91.7% (11)	100% (13)	100% (10)	0.711
clfA, clfB	Clumping factor A and B	100% (12)	100% (12)	100% (13)	100% (10)	-
cna	Collagen binding adhesin	58.3% (7)	25.0% (3)	61.5% (8)	70.0% (7)	0.151
ebh	Cell wall associated fibronectin-binding protein	100% (12)	100% (12)	69.2% (9)	70.0% (7)	**0.019**
ebps	Cell surface elastin-binding protein	100% (12)	100% (12)	100% (13)	100% (10)	-
eno	Enolase	100% (12)	100% (12)	100% (13)	100% (10)	-
fib	Fibrinogen-binding protein	100% (12)	100% (12)	100% (13)	100% (10)	-
fnbA	Fibronectin-binding protein A	100% (12)	100% (12)	100% (13)	100% (10)	-

fnbB	Fibronectin-binding protein B	83.3% (10)	83.3% (10)	69.2% (9)	100% (10)	0.315
map	Major histocompatibility complex class II analog protein	100% (12)	91.7% (11)	100% (13)	100% (10)	0.723
sasG	*S. aureus* surface protein G	33.3% (4)	58.3% (7)	69.2% (9)	70.0% (7)	0.259
sdrC	Serine-aspartate repeat protein C	100% (12)	100% (12)	100% (13)	100% (10)	-
sdrD	Serine-aspartate repeat protein D	91.7% (11)	91.7% (11)	92.3% (12)	100% (10)	1.000
vwb	Van Willebrand factor binding protein	100% (12)	100% (12)	100% (13)	100% (10)	-
Miscellaneous						
edinA, edinC	Epidermal cell differentiation inhibitor A+C	0	0	0	0	-
edinB	Epidermal cell differentiation inhibitor B	8.3% (1)	0	7.7% (1)	0	1.000
*setC**	Staphylococcal exotoxin-like protein	66.7% (8)[g]	91.7% (11)	100% (13)[g]	100% (10)	0.079
ssl6	Staphylococcal superantigen-like protein 6	8.3% (1)[h]	58.3% (7)[h]	38.5% (5)	30.0% (3)	0.077
ssl8	Staphylococcal superantigen-like protein 8	41.7% (5)[i]	91.7% (11)[i]	61.5% (8)	40.0% (4)[i]	**0.034**
ssl11	Staphylococcal superantigen-like protein 11	75.0% (9)	100% (12)[j]	53.8% (7)[j]	40.0% (4)[j]	**0.007**

[a] p values were calculated with Fisher's exact test; [b] significant difference between nasal colonisation and prosthesis infection (p=0.037), and between prosthesis infection and haematogenous OM (p=0.015); [c] significant difference between nasal colonisation and prosthesis infection (p=0.027) and prosthesis infection and sepsis (p=0.02); [d] significant difference between nasal colonisation and prosthesis infection (p=0.027) and prosthesis infection and sepsis (p=0.02); [e] significant difference between prosthesis infection and sepsis (p=0.011); [f] significant difference between nasal colonisation and prosthesis infection (p=0.047); [g] significant difference between prosthesis infection and haematogenous OM (p=0.039); [h] significant difference between nasal colonisation and prosthesis infection (p=0.027); [i] significant difference between nasal colonisation and prosthesis infection (p=0.027) and prosthesis infection and sepsis (p=0.02); [j] significant difference between prosthesis infection and haematogenous OM (p=0.015) and prosthesis infection and sepsis (p=0.03)

3.2.3 Enterotoxins & haemolysins

The most common enterotoxin gene group was the egc-cluster. In the group of prosthesis infections, the egc-cluster was less often present than in the other groups. The enterotoxin P was significantly more often found in this group. On the level of population genetics, it turned out that the egc-cluster was very specific for some CCs as they were found in all isolates of CC5, CC25, CC30, and CC45. Moreover, the EntP gene was significantly associated with CC7. The gene, which codes the toxic shock syndrome toxin, was found in two strains from nasal colonisation; while both strains belonged to CC30. This association was significant on clonal level.

All haemolysins were present in almost all strains and no group or CC-specific pattern could be observed.

3.2.4 Leucocidins & Exfoliative toxins

The γ-toxin coding gene was present in all the strains. The lukD and lukE genes were found significantly more often in the prosthesis infections group and were never found in combination with CC30, CC45, and CC398. The only isolate with a PVL gene originated from a nasal colonisation. Moreover, two of the three exfoliative toxin genes found were present in the same patient group of nasal colonisation.

3.2.5 Enzymes

The genes for aureolysin, glutamyl endopeptidase, staphylokinase, and staphylococcal complement inhibitor were detected in every strain or in almost every strain. The gene *chp* showed a very high frequency in the sepsis group. In contrast, it showed a very low frequency in the prosthesis group. The genes of the serine proteases A, B and E were most prevalent in the prosthesis group and less prevalent in the group of nasal and sepsis origin. The presence of those serine protease genes was significantly different between the clonal complexes. In detail none or only one isolate of CC30 and CC45 harboured the gene for serine protease A, B; whereas, the majority of CC was endowed with these genes. It was remarkable that all isolates of CC6, CC7 and CC30 were equipped with the serine protease E gene.

3.2.6 Exopolysaccharides & adhesins

All strains were equipped with either the cap5 or cap8 capsule coding gene. No group-specific distribution of the cap genes was evident. In all strains genes of the polysaccharide intracellular adhesin could be detected.

All isolates or almost all isolates were endowed with the adhesin genes *bbp, clfA, ebps, eno, fib, fnbA. map, sdrC, sdrD,* and *vwb*. An origin-specific distribution was observed for the gene *ebh* with a low frequency in groups of haematogenous osteomyelitis and sepsis. The CC30 stood out on the clonal level as this complex did not contain any genes for fibronectin-binding protein B, *S. aureus* surface protein G, and a low frequency of major histocompatibility complex class II analog protein gene.

3.2.7 Miscellaneous

In the group of epidermal cell differentiation inhibitors, just the *edinB* was rarely found and strongly associated to the CC25 and CC101, which are both included in the rare CCs group. The frequency of *setC* was reduced in the nasal isolates. All staphylococcoal superantigen-like proteins (ssls) were relatively rare in the sepsis group and most common in the group of prosthesis

infections. For the remaining isolate groups, the frequency of *ssls* was dependent on the single genes. Interestingly CC30 was missing several genes of this group (*edinA+B, setC, ssl6, ssl8*).

Table 11 Distribution of selected genes according to main clonal complexes.

Gene	Product	CC5	CC6	CC7	CC8	CC15	CC22	CC30	CC45	Rare CCs[a]	p[b]
	Regulation	**6**	**3**	**4**	**5**	**4**	**7**	**3**	**7**	**8**	
agrI	Accessory gene regulator allele I	16.7% (1)	66.7% (2)	100% (4)	80% (4)	75% (3)	57.1% (4)	0	85.7% (6)	87.5% (7)	**0.017**
agrII	Accessory gene regulator allele II	83.3% (5)	33.3% (1)	0	0	25% (1)	28.6% (2)	0	14.3% (1)	0	**0.008**
agrIII	Accessory gene regulator allele III	0	0	0	0	0	14.3% (1)	100% (3)	0	12.5% (1)	**0.002**
agrIV	Accessory gene regulator allele IV	0	0	0	20% (1)	0	0	0	0	0	0.404
	Resistance										
bla	β-lactamase	50.0% (3)	66.7% (2)	50.0% (2)	80.0% (4)	50.0% (2)	57.1% (4)	66.7% (2)	71.4% (5)	62.5% (5)	0.989
ermA	Erythromycin / clindamycin resistance gene A	16.7% (1)	0	0	0	25.0% (1)	0	0	14.3% (1)	12.5% (1)	0.923
ermB	Erythromycin / clindamycin resistance gene B	0	0	0	0	0	0	0	0	0	-
ermc	Erythromycin / clindamycin resistance gene C	0	0	0	20.0% (1)	0	0	0	0	0	0.404
fosB	Metallothiol transferase	83.3% (5)	100% (3)	0	60.0% (3)	75.0% (3)	57.1% (4)	100% (3)	14.3% (1)	37.5% (3)	**0.017**
mecA	Alternate penicillin binding protein 2, defining MRSA	16.7% (1)	0	0	0	0	14.3% (1)	0	14.3% (1)	12.5% (1)	1.000
sdrM	Transport-/ efflux protein	100% (6)	100% (3)	100% (4)	80.0% (4)	100% (4)	71.4% (5)	100% (3)	57.1% (4)	87.5% (7)	0.510
tetK	Tetracycline resistance gene K	0	0	0	0	0	28.6% (2)	0	0	0	0.322
tetM	Tetracycline resistance gene M	0	0	0	0	0	0	0	0	12.5% (1)	1.000
vanA	Vancomycin resistance gene	0	0	0	0	0	0	0	0	0	-

	Enterotoxins										
sea	Staphylococcal enterotoxin A	0	66.7% (2)	0	0	0	14.3% (1)	33.3% (1)	0	25.0% (2)	0.085
seb	Staphylococcal enterotoxin B	0	0	0	20.0% (1)	0	0	0	0	25.0% (2)	0.511
sec, sel	Staphylococcal enterotoxin C+L	0	0	0	20.0% (1)	0	28.6% (2)	0	42.9% (3)	0	0.279
sed, sej, ser	Staphylococcal enterotoxin D+J+R	16.7% (1)	0	0	40.0% (2)	25.0% (1)	14.3% (1)	0	14.3% (1)	0	0.646
see	Staphylococcal enterotoxin E	0	0	0	0	0	0	0	0	0	-
egc-cluster	Staphylococcal enterotoxin G+I+M+N+O+U	100% (6)	0	0	60.0% (3)	25.0% (1)	28.6% (2)	100% (3)	100% (7)	50.0% (4)	**0.000**
seh	Staphylococcal enterotoxin H	0	0	0	0	0	14.3% (1)	33.3% (1)	0	12.5% (1)	0.699
sek, seq	Staphylococcal enterotoxin K+Q	0	0	0	0	0	0	0	0	25.0% (2)	0.540
tst1	Toxic shock syndrome toxin (TSST)-1	0	0	0	0	0	0	66.7% (2)	0	0	**0.006**
entP	Enterotoxin P	16.7% (1)	0	100% (4)	0	0	0	0	14.3% (1)	0	**0.000**
	Haemolysins										
hla	α-toxin	100% (6)	100% (3)	100% (4)	100% (5)	100% (4)	100% (7)	100% (3)	100% (7)	100% (8)	-
hlb	β-toxin	83.3% (5)	66.7% (2)	100% (4)	80.0% (4)	100% (4)	100% (7)	100% (3)	85.7% (6)	100% (8)	0.871
hld	δ-toxin	100% (6)	100% (3)	100% (4)	100% (5)	100% (4)	100% (7)	100% (3)	100% (7)	100% (8)	-
	Leucocidins										
lukF, hlgA	γ-toxin	100% (6)	100% (3)	100% (4)	100% (5)	100% (4)	100% (7)	100% (3)	100% (7)	100% (8)	-
lukF-PV	Panton-Valentin-leucotoxin	0	0	0	0	0	0	0	0	12.5% (1)	1.000
lukD, lukE	Leucocidin D, E component	83.3% (5)	100% (3)	100% (4)	60.0% (3)	75.0% (3)	71.4% (5)	0	14.3% (1)	50.0% (4)	**0.022**

	Exfoliative toxins	CC5	CC6	CC7	CC8	CC15	CC22	CC30	CC45		p[a]
etA	Exfoliative toxin A	0	0	0	0	0	0	0	14.3% (1)	0	0.830
etB	Exfoliative toxin B	0	0	0	0	0	0	0	0	0	-
etD	Exfoliative toxin D	0	0	0	0	0	0	0	0	25.0% (2)	0.540
	Enzymes										
aur	Aureolysin	100% (6)	100% (3)	100% (4)	100% (5)	100% (4)	100% (7)	100% (3)	100% (7)	100% (8)	-
chp	Chemotaxis inhibitory protein (CHIP)	50.0% (3)	33.3% (1)	0	40.0% (2)	50.0% (2)	57.1% (4)	100% (3)	85.7% (6)	75.0% (6)	0.131
splA, splB	Serine protease A, B	83.3% (5)	100% (3)	100% (4)	60.0% (3)	75.0% (3)	71.4% (5)	0	14.3% (1)	50.0% (4)	**0.022**
splE	Serine protease E	0	100% (3)	100% (4)	40.0% (2)	50.0% (2)	42.9% (3)	100% (3)	0	37.5% (3)	**0.001**
sspA, sspB	Glutamyl endopeptidase	100% (6)	100% (3)	100% (4)	100% (5)	100% (4)	100% (7)	100% (3)	100% (7)	100% (8)	-
sak	Staphylokinase	100% (6)	66.7% (2)	100% (4)	100% (5)	75.0% (3)	57.1% (4)	100% (3)	85.7% (6)	75.0% (6)	0.478
scn	Staphylococcal complement inhibitor	100% (6)	100% (3)	100% (4)	100% (5)	100% (4)	100% (7)	100% (3)	85.7% (6)	100% (8)	0.830
	Exopolysaccharides										
cap5	Capsular polysaccharide 5	83.3% (5)	0	0	60.0% (3)	25.0% (1)	42.9% (3)	0	57.1% (4)	62.5% (5)	0.081
cap8	Capsular polysaccharide 8	16.7% (1)	100% (3)	100% (4)	40.0% (2)	75.0% (3)	57.1% (4)	100% (3)	42.9% (3)	37.5% (3)	0.081
icaA, D, C	Polysaccharide intracellular adhesin	100% (6)	100% (3)	100% (4)	100% (5)	100% (4)	100% (7)	100% (3)	100% (7)	100% (8)	-
	Adhesins										
bbp	Bone sialoprotein-binding protein	100% (6)	100% (3)	100% (4)	100% (5)	75.0% (3)	100% (7)	66.7% (2)	100% (7)	100% (8)	0.079

clfA, clfB	Clumping factors A and B	100% (6)	100% (3)	100% (4)	100% (5)	100% (4)	100% (7)	100% (3)	100% (7)	100% (8)	-
cna	Collagen binding adhesin	16.7% (1)	66.7% (2)	0	60.0% (3)	25.0% (1)	57.1% (4)	66.7% (2)	85.7% (6)	75.0% (6)	0.069
ebh	Cell wall associated fibronectin-binding protein	100% (6)	100% (3)	100% (4)	80.0% (4)	100% (4)	71.4% (5)	100% (3)	57.1% (4)	87.5% (7)	0.510
ebps	Cell surface elastin-binding protein	100% (6)	100% (3)	100% (4)	100% (5)	100% (4)	100% (7)	100% (3)	100% (7)	100% (8)	-
eno	Enolase	100% (6)	100% (3)	100% (4)	100% (5)	100% (4)	100% (7)	100% (3)	100% (7)	100% (8)	-
fib	Fibrinogen-binding protein	100% (6)	100% (3)	100% (4)	100% (5)	100% (4)	100% (7)	100% (3)	100% (7)	100% (8)	-
fnbA	Fibronectin-binding protein A	100% (6)	100% (3)	100% (4)	100% (5)	100% (4)	100% (7)	100% (3)	100% (7)	100% (8)	-
fnbB	Fibronectin-binding protein B	100% (6)	100% (3)	100% (4)	60.0% (3)	100% (4)	85.7% (6)	0	85.7% (6)	100% (8)	**0.018**
map	Major histocompatibility complex class II analog protein	100% (6)	100% (3)	100% (4)	100% (5)	100% (4)	100% (7)	66.7% (2)	100% (7)	100% (8)	0.128
sasG	*S. aureus* surface protein G	83.3% (5)	100% (3)	0	60.0% (3)	50.0% (2)	100% (7)	0	57.1% (4)	37.5% (3)	**0.005**
sdrC	Serine-aspartate repeat protein C	100% (6)	100% (3)	100% (4)	100% (5)	100% (4)	100% (7)	100% (3)	100% (7)	100% (8)	-
sdrD	Serine-aspartate repeat protein D	100% (6)	100% (3)	100% (4)	100% (5)	75.0% (3)	85.7% (6)	66.7% (2)	100% (7)	100% (8)	0.256
vwb	Van Willebrand factor binding protein	100% (6)	100% (3)	100% (4)	100% (5)	100% (4)	100% (7)	100% (3)	100% (7)	100% (8)	-
	Miscellaneous	**CC5**	**CC6**	**CC7**	**CC8**	**CC15**	**CC22**	**CC30**	**CC45**		
edinA, edinC	Epidermal cell differentiation inhibitor A+C	0	0	0	0	0	0	0	0	0	-
edinB	Epidermal cell differentiation inhibitor B	0	0	0	0	0	0	0	0	25.0% (2)	0.540
setC	Staphylococcal exotoxin-like protein	100% (6)	100% (3)	100% (4)	100% (5)	100% (4)	100% (7)	0	71.4% (5)	100% (8)	**0.000**
ssl6	Staphylococcal superantigen-like protein 6	0	33.3% (1)	100% (4)	60.0% (3)	50.0% (2)	57.1% (4)	0	0	25.0% (2)	**0.004**

ssl8	Staphylococcal superantigen-like protein 8	83.3% (5)	100% (3)	100% (4)	60.0% (3)	75.0% (3)	71.4% (5)	0	14.3% (1)	50.0% (4)	**0.022**
ssl11	Staphylococcal superantigen-like protein 11	83.3% (5)	100% (3)	100% (4)	60.0% (3)	75.0% (3)	71.4% (5)	100% (3)	14.3% (1)	62.5% (5)	0.072

[a] CCs with fewer than three isolates (CC1, CC25, CC101, CC121, CC398) were summarised in one group

[b] p values were calculated with Fisher's exact test

3.3 Bacterial origin did not determine differences in cytotoxicity

To determine the influence of the described genotypic differences on the phenotype, additional functional assays were performed.

To exclude differences in growth behaviour as an influencing factor on other phenotypic traits, the bacterial growth was monitored (s. Figure 10). One strain stood out due to its slowed growth which was visible after overnight incubation on Columbia blood agar plates as well as in the respective growth curve. However, the vast majority of strains showed a normal, group-unrelated growth dynamic.

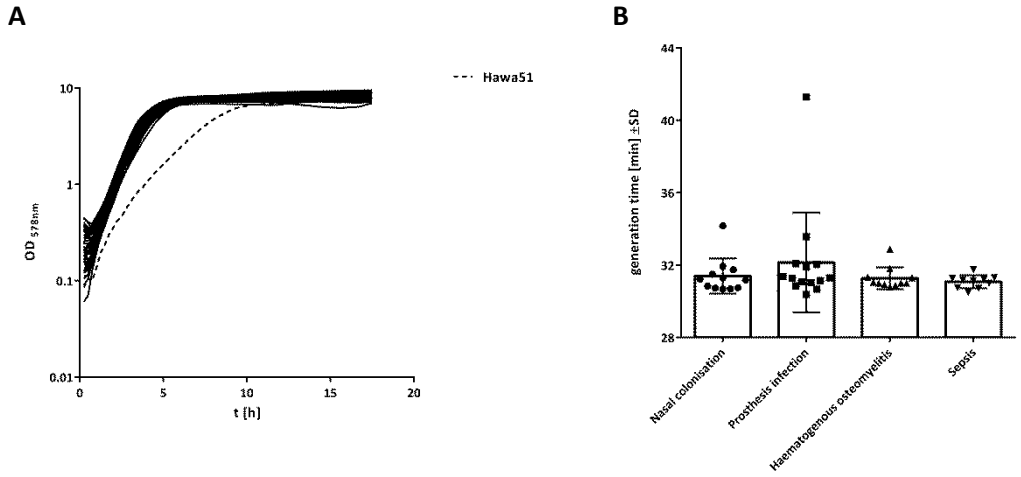


Figure 10 Growth dynamic of *S. aureus* patient isolates. Clinical *S. aureus* isolates were monitored over 16 h by photometrical measurement of the optical density. **A** The increase of the optical density over time shows a homogenous growth for all strains except the strain Hawa51. **B** When strains were categorised by the infectious situation they were isolated from, no group specific difference could be detected. The differences between the isolate groups were analysed by a one-way ANOVA test with Tukey's multiple comparisons test.

Comparably to an infection in the human body, different stages of the infectious process were simulated by testing the bacterial isolates in the following experiments (s. Figure 11). The infection was initiated with the first contact of bacterium with host cell and subsequent uptake of the pathogen by the latter. In the invasion assay the number of infected cells was determined. Furthermore, the approximate bacterial load was estimated by detection of the mean fluorescence intensity within the gate of the invaded cells. When the quantity of invaded cells was compared on the level of the isolate groups, no significant differences were visible. The same was true when groups were compared regarding the bacterial load. All groups showed a wide distribution of the individual strains. In the course of infection, staphylococci usually leave the former invaded host cell. These actions cause cell death [285] which is assessed by the cytotoxicity assay and haemolysis assay. Both assays illustrate the effect of secreted virulence factors on bone cells and erythrocytes. Similar to the invasion assay, all groups consisted of a very diverse composition of strains differing in their cytotoxic abilities. Prior to the secretion of virulence factors, the

expression of certain genes was required. The central regulator of pathogenicity was *agrA* which was therefore quantified on the gene expression level. AgrA is i.a. controlling the expression of the RNA effector molecule *rnaIII* which in turn regulates *psmα* and *hla* expression. Taken together the mRNA quantity of those genes helped to sketch a picture of the regulation of virulence for a single strain. As all genes are regulated in a quorum sensing dependent manner, the gene expression could be determined at defined timepoints of incubation in a bacterial culture. The gene expression analysis revealed no group-specific differences for *agrA, rnaIII, psmα,* and *hla*. However, for *psmα* and *hla*, relatively homogenous low expression was monitored in the group of haematogenous osteomyelitis.

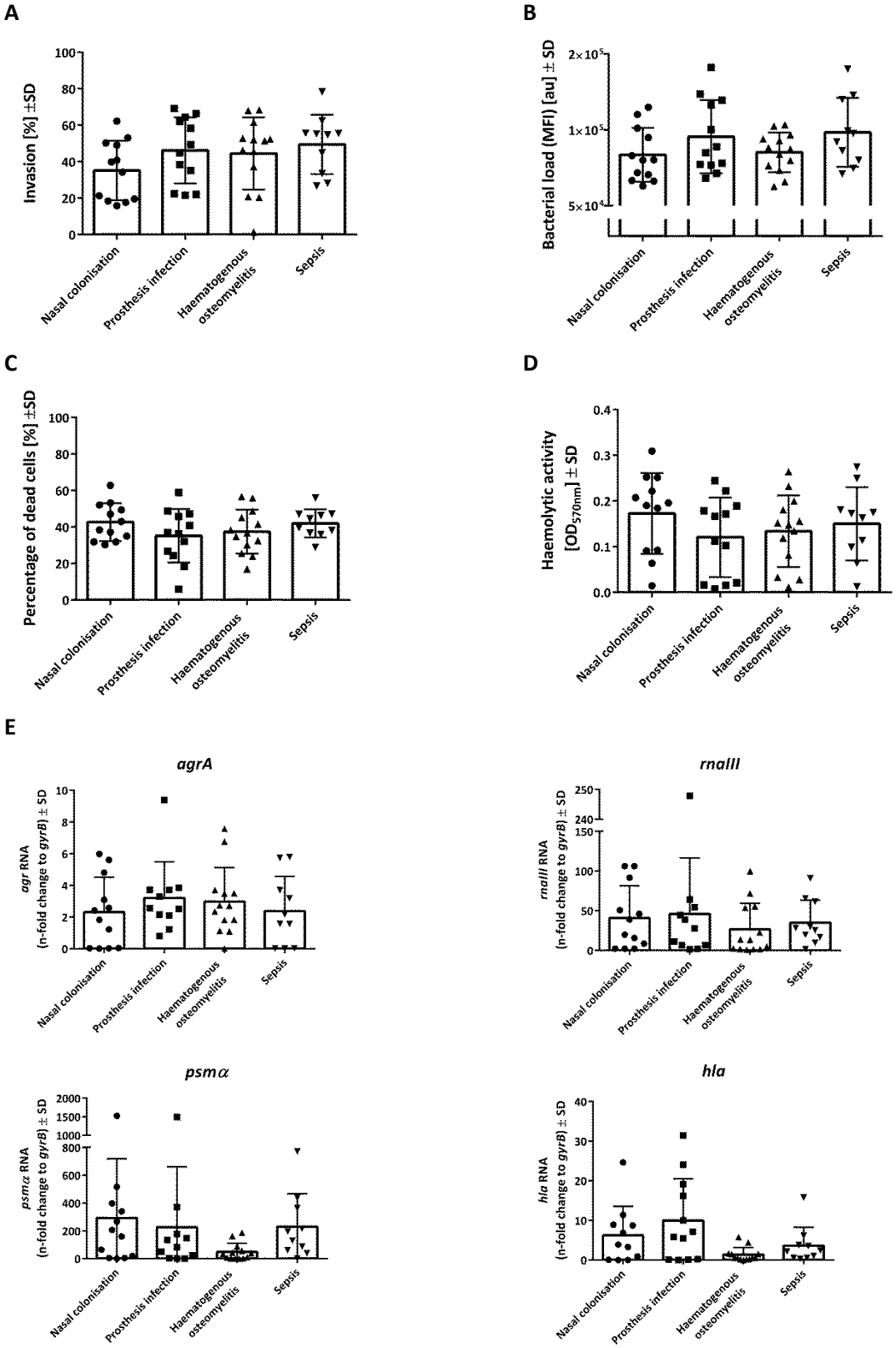


Figure 11 Group affiliation was not associated with a specific phenotype or gene expression level of selected central virulence regulators. A The osteoblast cell line hFOB 1.19 was infected with FITC-stained *S. aureus* and the quantity of intracellular bacteria was determined by flow cytometry. **B** The approximate bacterial load of the osteoblast with the stained staphylococci was assessed by measurement of the mean fluorescence intensity of the invaded host cells. **C** The osteoblasts were infected with clinical *S. aureus* isolates and the rate of dead cells was quantified 24h p.i. flow cytometrically. **D** The haemolytic ability of the strains was assessed by the co-incubation of bacterial culture supernatants with sheep blood erythrocytes. Secreted haemolytic compounds in the supernatant destroyed the erythrocytes which released haemoglobin. The free haemoglobin was quantified with a

spectrophotometer at a wavelength of 570nm. **E** Gene expression of *S. aureus* clinical isolates. All isolates were cultivated in BHI for 4 h (*agrA, rnaIII*) and 6 h (*psmα, hla*), respectively. The RNA was isolated and reversely transcribed into complementary DNA (cDNA) in order to perform qPCR. The slow growing strain Hawa51 was not included in the graph for the gene expression data due to its changed growth dynamic. The bars and whiskers represent the means ±SD of at least three independent experiments in duplicate. The differences between the isolate groups were analysed by a one-way ANOVA test with Tukey's multiple comparisons test.

3.4 Investigation of group-unrelated correlations

As no group-specific pattern could be detected in the previously described experiments, the pairwise correlation of all data sets was calculated (s. Figure 12). The expression of all investigated genes was positively correlated, whereas the most significant correlations were found between *agr* and *rnaIII* ($p<0.001$, $r= 0.55$), *rnaIII* and *psmα* ($p<0.001$, $r= 0.67$), and *psmα* and *hla* ($p<0.001$, $r= 0.58$). The selected genes were key factors for the staphylococcal virulence and were therefore engaged in the same or overlapping processes. As these processes usually led to the death of the host cell, the detected positive correlation between cytotoxicity and *hla* ($p=0.001$, $r=0.46$), *rnaIII* ($p<0.001$, $r= 0.53$) and *psmα* ($p<0.001$, $r= 0.61$) was reasonable. The cytotoxic ability was additionally correlated with haemolysis ($p=0.013$, $r=0.37$), which indicates that both mechanisms were driven by shared actions. This was underlined by the positive correlation of haemolysis to the same genetic factors as seen for cytotoxicity (*hla:* $p=0.008$; $r=0.38$, *rnaIII:* $p=0.001$; $r= 0.49$, *psmα:* $p<0.001$, $r= 0.52$). Surprisingly, cytotoxicity and haemolysis showed a negative correlation to the invasiveness ($p=0.026$ $r=-0.33$) ($p=0.004$ $r=-0.42$) which suggested that these were opposing characteristics. It was observed that invasion and bacterial load were strongly correlated ($p<0.001$, $r= 0.82$) and shared the same pattern for correlation with the other factors (s. Table S 3). A correlation of the generation time with any of the other characteristics could not be confirmed (s. Table S 3).

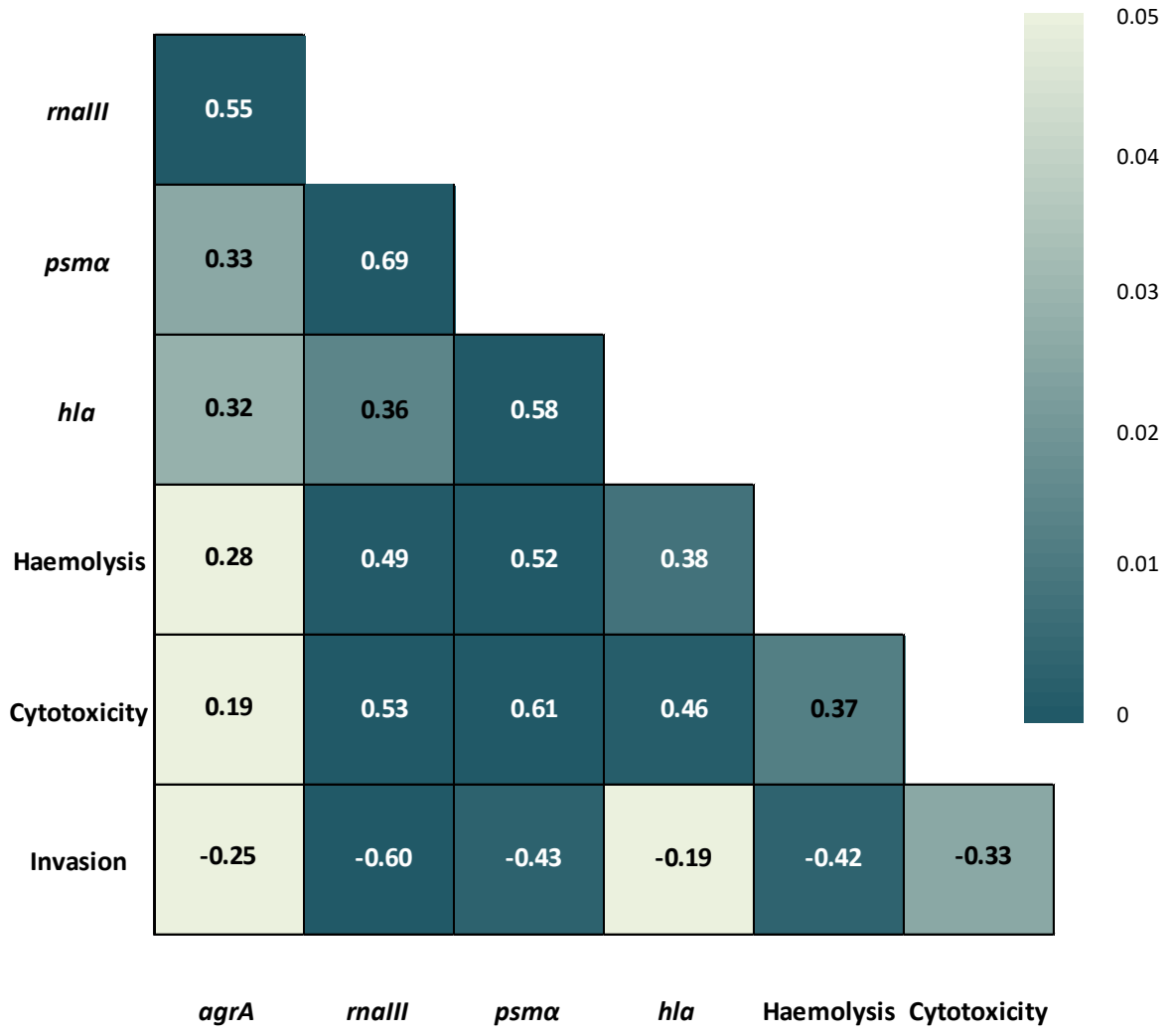


Figure 12 Non-parametric correlation analysis of all measured parameters. The two-sided p-values are represented by a colour coding scheme (heat map) where the p-value decreases from light (high p-value, low significance) to dark blue (low p-value, high significance). Spearman's rank correlation coefficient is displayed as numbers. Data for the strain Hawa51 (SCV phenotype) are excluded from correlation analysis. The full correlation analysis is given in Table S 3.

3.4.1 High bacterial cytotoxicity and low invasiveness are beneficial for persistence inside host cells

S. aureus is able to hide from the immune system within cells of the host for longer times by downregulating the gene expression for general metabolism and toxins [248]. This persistence ability is an important virulence trait and was therefore assessed with a long-time persistence model (s. Figure 13). Special attention was paid to a possible association of virulence, namely high cytotoxicity and haemolysis, and persistence. For this reason, four high and three low cytotoxic strains were selected for infection of osteoblasts. The infected cells were lysed at previously

defined timepoints, and the lysate was plated to define the bacterial number. In general, all strains can persist regardless of their cytotoxic characteristic. However, the later the timepoint for lysis, the higher the difference between bacterial numbers of low and high cytotoxic strains; whereas, low cytotoxic strains persisted in significantly higher numbers. This difference is especially pronounced on day 7 after infection. SCVs were regarded as a special adaptation to the intracellular environment during persistence. The percentage of this phenotype was significantly raised for the low cytotoxic strains at the last day of the experiment. Additionally, the induced immune reaction was quantified on behalf of the levels of Chemokine (C-C motif) ligand 5 (CCL5 / RANTES).

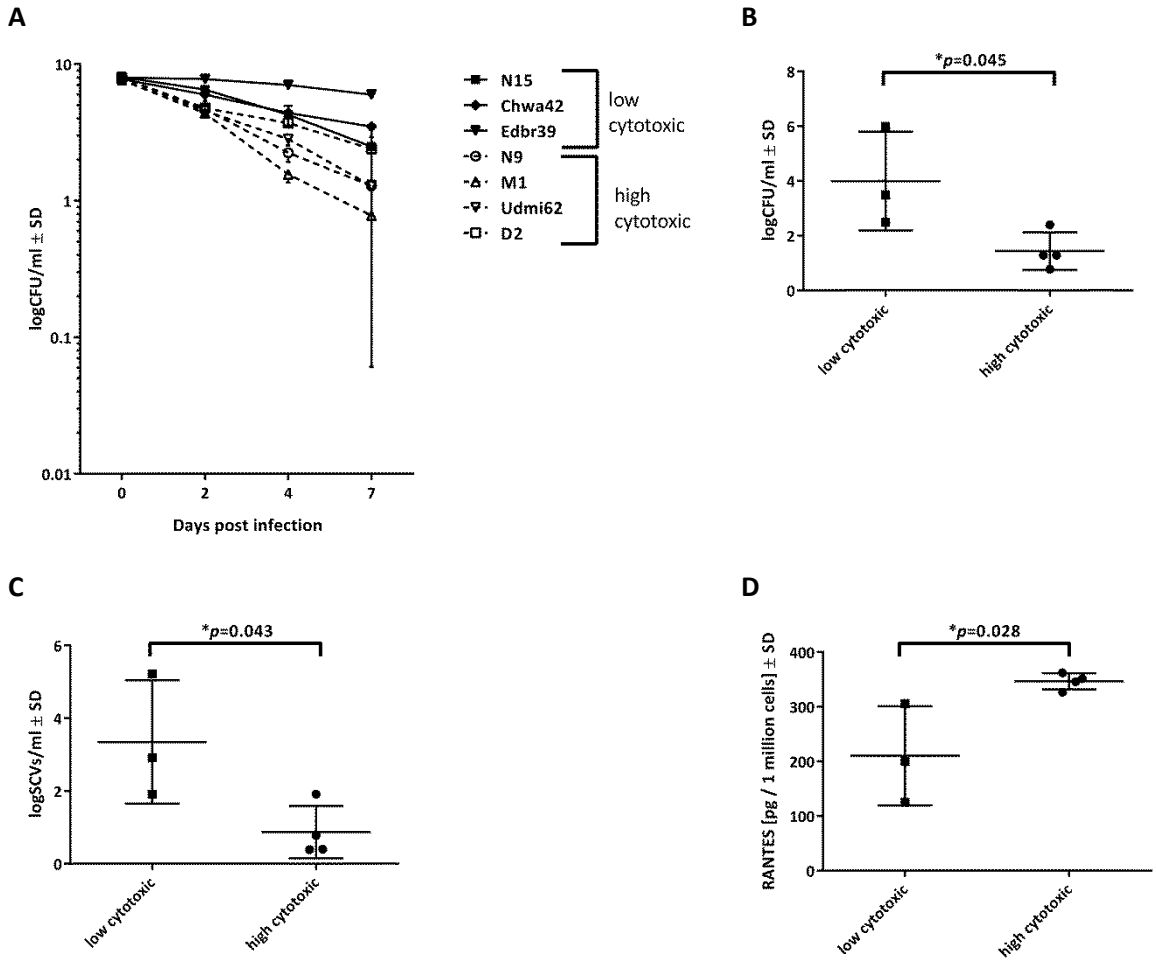


Figure 13 Long-term persistence of low and high cytotoxic strains in osteoblasts. A Intracellular bacteria recovered from infected osteoblasts for four high cytotoxic strains (black lines) and three low cytotoxic strains (gray lines) as a function of time p.i.. **B** Intracellular bacteria recovered from infected osteoblasts with high and low cytotoxic strains at day 7 p.i.. **C** SCVs recovered at day 7 p.i.. **D** RANTES levels were measured in cell culture supernatant of infected osteoblasts after 24-h p.i. using ELISA test. All results represent the means of of at least three independent experiments ± SD. The difference between low and high cytotoxic strains was analysed by an unpaired T-test.

The secretion of CCL5 was reduced in a significant grade in the cells which were infected with low cytotoxic strains. In summary, the low cytotoxic strains persisted in higher numbers over the course of infection by transition of a higher percentage of bacteria to the SCV phenotype which caused a reduced host immunological reaction compared to high cytotoxic strains.

3.4.2 Low cytotoxic strains persist in higher numbers in the murine sepsis model than high cytotoxic strains

A murine sepsis model was performed to evaluate the transferability of the findings from the cell culture infection experiment to a mammalian organism. Two *S. aureus* strains, which were differing in their cytotoxic ability, were selected to be tested. The results are summarised in Figure 14. Within 5 days p.i. both groups of mice developed symptoms of sepsis mainly characterised by a severe weight loss during that time. Surprisingly, the infection with the low cytotoxic strain Chwa42 led to a higher mortality. However, the survival rate between both groups did not differ significantly. The mice were sacrificed after 5 weeks in the phase of a chronic infection. The homogenisation and subsequent plating of the leg bones showed a significant higher bacterial recovery for the low cytotoxic strain Chwa42. In a similar murine infection experiment, blood was withdrawn from the mice 3 days p.i. to analyse the immune response. The quantification of CCL5 (RANTES) showed a higher cytokine level for the high cytotoxic strain D2. These results suggest that low cytotoxic strains are less immunogenic and therefore worse cleared from their host immune system, which leads to a better persistence in the mammalian body.

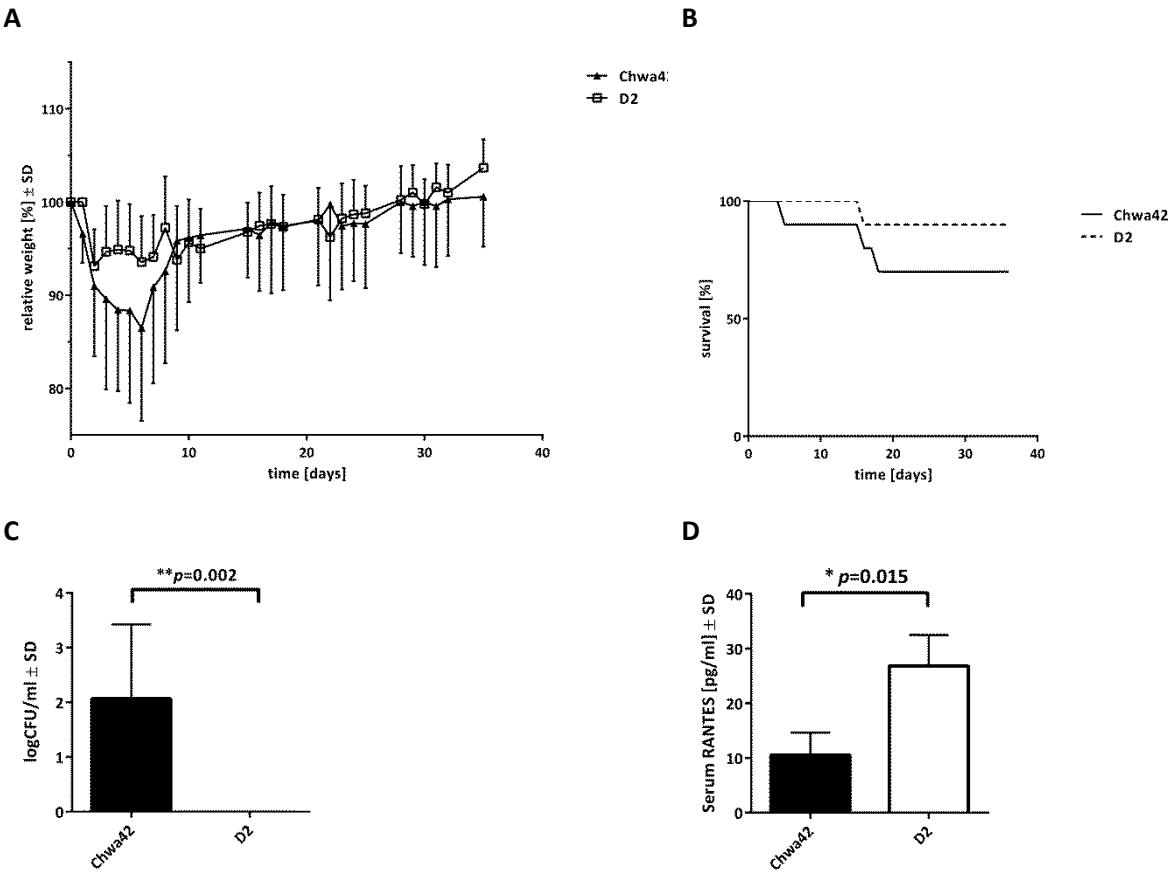


Figure 14 High-cytotoxic strain induced high inflammation and fast clearance by the host in the mouse sepsis model. C57/BL6 mice were infected with Chwa42 (low cytotoxic) or D2 (high cytotoxic) strains for 6 weeks. **A** The relative weight of the mice decreased a few days after infection as a symptom of the development of sepsis. After survival of the septic condition, the weight constantly increased. **B** Survival curves of the infected mice with Chwa42 (n=8) and D2 (n=10). No differences were observed by a long-rank (Mantel Cox) test. **C** The bacterial loads within the hind limbs were analysed after 6 weeks p.i.. Bar and whiskers represent mean±SD. Statistical analysis was performed with an unpaired t-test comparing the bacterial load in hind limbs (*p=0.0021). **D** RANTES levels in serum were measured after 3 days p.i.. Statistical analysis was performed using the unpaired t-test comparing abundance of RANTES in the serum (*p ≤= 0.0015).

4. Discussion

S. aureus' relation to its human host varies in many factors namely the grade of disadvantage for the host (commensalism to infection), the quality of infection (superficial to deep tissue), the location of infection (skin, heart, bones), and the duration of an infection (acute to persistent) [4]. The combination of the listed characteristics creates a unique ecological niche which requires respective adaptations for survival. It was therefore proposed that only strains with specific genotype and phenotype are able to occupy one specific niche in the form of an infection. The hypothesis included the assumption that bacteria, which are adapted to the same infectious situation, would show similar characteristic patterns. Based on this theory, a collection with four groups of strains was established. The groups were defined by their origin, which was associated to blood (haematogenous osteomyelitis, sepsis), to bone (prosthesis infection, haematogenous osteomyelitis), to blood and bone (haematogenous osteomyelitis), or to none of those criteria (nasal isolates). The central aim was to identify single factors or patterns which were linked to bacterial infection and survival within the blood stream or the skeletal apparatus.

By comparing characteristics of the patient groups, group-specific differences came to light. The mortality rate between the patient groups differed significantly - where the more severe infections (haematogenous osteomyelitis and sepsis) had a higher probability of a fatal outcome. Concurrently, these two groups showed higher rates in comorbidities per patient and a raised patient age. Regarding the fact that the staphylococcal strains of those groups also included low cytotoxic strains, the higher case fatality rates could probably be attributed to the poor overall condition of the patients suffering from haematogenous osteomyelitis and sepsis. It was hypothesised by Young that patient factors like comorbidities create a "distinctive selection pressure" which promotes the induction of disease-specific mutations on the bacterial side [286]. Therefore, the role of the host in the development of an infectious disease was not underestimated and was highlighted in the past by several studies [287]. When the host's immune response is extensively compromised, even formerly saprophytic and apathogenic staphylococci act in a pathogenic manner [288]. The data on association of clonal complexes to specific diseases or virulence traits are ambiguous. Some studies found a prevalence of single clonal complexes in invasive diseases [289-292], whereas, other studies couldn't link the clonal background to a specific pathology [287, 293]. In particular strains of the CC30 were found to be the major causative agent of severe invasive diseases [289-291] which could not be confirmed in this study. One reason for this opposing observation could be the low general prevalence of CC30 in the present data set as also described by Feil et al. [292]. In our sample strains isolated from severe invasive infections (haematogenous osteomyelitis and sepsis) showed a strong association to

CC22. This finding was consistent with previous studies which identified prevalently strains with this clonal background in haematogenous osteomyelitis [287].
Strains of the CC398 were first noticed as their high colonisation rate of livestock was discovered which represents a zoonotic reservoir of MRSA [282, 294-297]. However, there was evidence indicating a human origin of our isolates. As described previously [298, 299], the endowment with the type 3 φSa3 prophage – associated genes (*chp*, *scn*) enable the expression of innate immune modulatory factors which is regarded as an adaptation to humans.

On the level of individual genes, the enterotoxin P showed a higher prevalence in the group of prosthesis infections. This was surprising, as the endowment with *entP* was described as a predictive factor for a severe course of infection [300, 301].
Interestingly, a lot of relatively rare toxin genes, including *tst, eta, etd, lukF-PV*, were found in the group originating from nasal colonisation. This apparent discrepancy between asymptomatic colonisation and potentially highly cytotoxic strains was observed in the past [302]. Laabei et al. explained this discovery with the reduced ability of high toxic strains to survive in the serum and therefore their inability to cause bacteraemia. Moreover, Shallcross et al. showed in their meta-analysis study that PVL was not associated with invasive infections [303].
The presence of the leucocidin genes *lukD* and *lukE* was not described to be strictly associated to implant infections. Usually, the frequency of those genes is around 65% in bone-related infections [304, 305]. The encoded leucocidin ED is toxic to phagocytes which are recruited to the site of infection [306]. Although this function is certainly favourable for the establishment of a prosthesis infection, it cannot explain the lower frequency of this gene combination in the group of haematogenous osteomyelitis.
Even though genes of the *spl* operon are highly immunogenic [307, 308], only a supportive function for the disease development has been proven in previous studies [307, 309]. A substrate specificity of the single serine proteases is suspected [310, 311], but a related specific correlation with pathologies is not identified yet [307]. The high prevalence of *splA* / *splB* in prosthesis infections has not been described until now, and its mechanistic role needs to be elucidated in future research.
The plasminogen activator staphylokinase, which is coded by the gene *sak,* was found more often in context with diseases in contrast to the group of asymptomatic colonisations. This result conforms with previous studies [312, 313]. The elevated frequency of *sak* could be explained by its ability to promote bacterial dissemination [314] and to prevent opsonisation by the host immune system [315].
The association of the *β-lactam* resistance operon with sepsis and *fosB* with mortality was already shown [287] and supports our finding of an elevated frequency of those genes in the group of

sepsis isolates. However, if the mere existence of the resistance genes is causative for observed course and severity of the disease cannot be said with certainty.

When the frequency of single genes is analysed, the highly clonal nature of *S. aureus* should be considered [316]. Because of this population structure, some gene combinations are specific for a matching CC, and the correlation of an observed effect to a single gene can also be caused by an associated gene [317]. This phenomenon is known as hitchhiker effect or linkage disequilibrium and is well documented for *S. aureus* [316-318]. Two challenges arise from this condition: first, our data were not corrected for this effect by comparing only inter-clonal variations. Second, only selected genes were checked for their presence in the used isolates, so it is possible that the crucial genetic factor was not documented by the method used. Prospectively, the use of whole genome sequencing is promising to detect differences among all genes and to identify putative virulence determinants which have been overlooked until now. Especially with regard to the fact that staphylococcal evolution is mainly driven by point mutations [292, 302] and repeat number variations [316], it is essential to analyse the genome in its full complexity. The application of RNA sequencing (RNA-seq) and proteomics would complete the picture on the bacterial side of the infection.

Although we identified some trends in the relation of clonality and pathology, this is not enough to explain the development, or non-development of specific infections. Even though the distribution of the sampled strains among the CCs corresponds to larger national and international studies [287, 290, 292, 319, 320], our dataset is limited to fewer than 50 isolates from a single hospital. As a consequence of the relatively small sample size, only very pronounced differences could be detected. The intention of this work was not to analyse the epidemiological distribution of *S. aureus* in dependence of its clonal background, but rather to draw a picture of the connection of bacterial genotype; phenotype and caused pathology - in order to identify distinct virulence mechanisms and strategies.

Based on the examination of the strains' genetic constitution, functional assays were used to evaluate the bacterial phenotype in different situations. When the results of the conducted experiments were examined, a striking pattern was visible: every group of isolates harbors strains which rank within a wide spectrum of the measured trait. There are several possible reasons for this kind of distribution. One option is that the strains are similar in vivo in the actual infectious situation in the patient, but the model used cannot rebuild this setting. This could be explained by a genotype-by-genotype specificity [321] of the tested strains where the observed pathology is generated by the mutual reaction of one specific bacterial genotype with a specific host genotype. This kind of relationship can be difficult to imitate in an experimental setting. It is also possible that the strains are as different as the exemplary assays show and that "virulent and non-virulent

strains are not fundamentally different from each other" [319] when it comes to causing a particular disease. The combination of both approaches results in a theory where the strains are as different as it is observed in the functional assays but due to the individual interplay with different hosts, they produce a uniform clinical picture. This theory includes two assumptions: first, that different strategies lead to the same result, and second, that the combination of host and pathogen is important. The dynamic concept, which forms the basis of the pathogen definition, harmonises with this idea [259].

The link between genetic constitution and the bacterial phenotype is gene expression. To elucidate the regulation of virulence, the expression of selected genes was examined. The amount of expressed quorum sensing accessory gene regulator (*agr*) and its effector molecule RNAIII per strain in dependence of the isolate group displayed a similar pattern as seen for the functional assays. The low expression of *agr* and even the complete dysfunction of the gene were shown to be conducive for the development of infections, especially in the hospital setting [322]. Moreover, *agr* dysfunctional strains were also found as colonisers of healthy people [323]. Apart from that, a high expression of *agr* is crucial for diseases like skin infections [324] and clinical research indicates an important role for the acute infection [254]. Therefore, it cannot be assumed that a certain degree of *agr* expression is advantageous for an infection. Instead, the correlation is highly specific for additional factors like mode of bacterial acquisition and course of infection. It appears that the applied group classification is not precise enough to include all those decisive circumstances. According to the fact that upon activation of transcription of *agrA*, the locus coding for *rnaIII* is activated simultaneously [325], a similar relation between the gene expression levels for both genes was expected in all strains. The pattern of strain distribution was different for the toxin gene *hla* and for the gene coding the cytolytic peptide *psmα*. For both genes a lower expression was measured when the strains originated from haematogenous osteomyelitis. Contrary to expectation, a high expression of those virulence associated genes is associated with a better prognosis for the patient [326-328], which could explain the higher expressing strains from the low-mortality groups of nasal and prosthesis origin.

The initially expected major differences between the isolate groups were not observed by means of analysis of genotype, gene expression, nor functional phenotype. The increase of the quantity of tested bacterial isolates would likely have resulted in significant differences in some experiments. However, it could not hide the fact that a broad variety of differently shaped bacteria are able to cause a similar pathological picture. It was therefore investigated if strains share unique patterns of virulence independent from their background. In order to recognise connected characteristics, correlation analysis was used. This analysis showed a positive correlation of the

expression of all four tested genes. This correlation is in accordance with the established model of gene regulation where AgrA as a central regulator promotes the transcription of *rnaIII* [329] and *psmα* [90]. The small regulatory RNA RNAIII activates the transcription and translation of *hla* amongst other virulence-related genes [325, 330]. As both encoded proteins α-toxin and PSMα form pores on different host cells [331-334], the secretion of those toxins is linked to high haemolysis and cytotoxicity. Remarkably, all those listed factors were negatively correlated with the ability to invade cells and the number of intracellular bacteria. This fact suggests the existence of two distinct modes of operation for the examined sample. High cytotoxicity excludes high invasiveness and vice versa.

To investigate the importance and characteristics of these bacterial infection strategies, an osteoblast cell culture infection model was used to examine selected strains during long time persistence. For this experiment strains were chosen from the extreme ends of the cytotoxic spectrum, that is very low and very high cytotoxic strains with opposed invasive characteristics. Even though similar numbers of bacterial colonies were recovered at earlier time points, the difference of low and high cytotoxic strains increased over the time of infection. At the endpoint, 7 d after infection, low cytotoxic strains persisted significantly better compared to their high cytotoxic counterparts. Nevertheless, it was possible for all the strains to persist in this simplified infection model. This finding leads to the assumption that the degree of cytotoxicity does not influence the general ability to infect and survive within host cells. However, the success of the infection rises contrary to the strain specific cytotoxicity level. An obvious reason could be found in the reduction of host cells in their function as protective compartments by toxin-mediated destruction of these very structures. This effect can probably be attributed to the limitations of the used model: a one-dimensional layer of a single cell type without professional immune cells. For a more general explanation of the success of low cytotoxic staphylococci, the host immune response needs to be considered. The increased secretion of toxins by the high cytotoxic strains caused a stronger immunological reaction which is illustrated by higher RANTES-levels of the corresponding host cells. The observations of the cell culture model were confirmed and underlined in the complex murine infection model. It was shown that if the infection was conducted in an organism with a fully functional mammal immune system, the high cytotoxic strain was completely eliminated from the body during the course of infection. In contrast, the low cytotoxic strain induced a weaker immune reaction and was able to persist inside the bone.

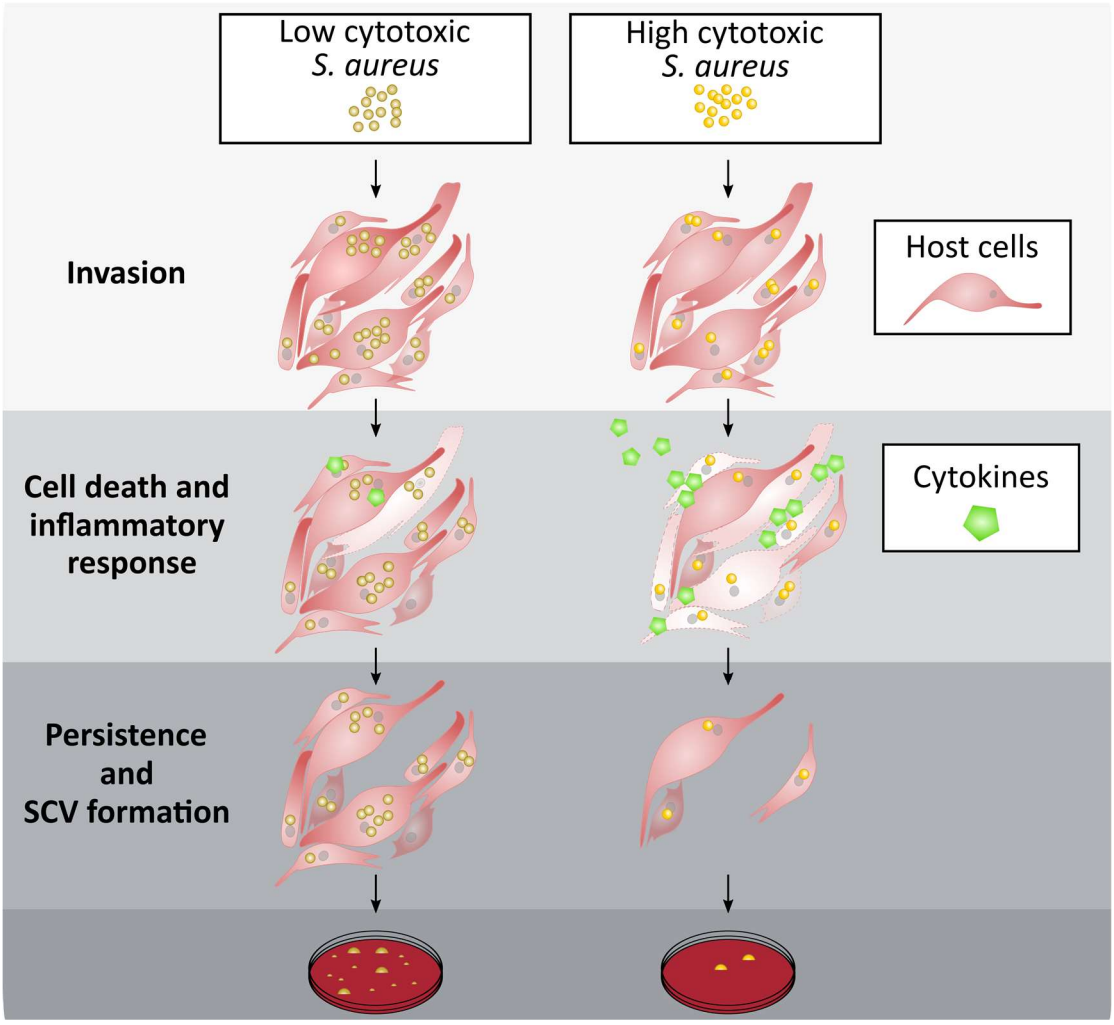


Figure 15 In the course of infection, low cytotoxic strains have an advantage over high cytotoxic strains due to their higher invasion, lower cytotoxicity, and better persistence inside the host cells. High and low cytotoxic strains represent two distinct infection strategies. High cytotoxic strains have a generally higher level of *agr* and *rnaIII* regulated toxin gene expression (e.g. *hla* and *psmα*). Induced by the damaging effects of the secreted toxins, the host cells release corresponding amounts of cytokines. Some cytokines can further increase the bacterial induced cell death. The combination of high levels of staphylococcal toxins and host cytokines lead to a pronounced reduction of intact host cells in infections with high cytotoxic strains. As host cells serve as sheltering compartment for long-time persistence, low cytotoxic *S. aureus* strains can persist in higher numbers while exhibiting the SCV phenotype more often. Measured by the recovery of living intracellular bacteria, "silent" infection with low cytotoxic strains is the more successful strategy in a mono-cell culture infection model. Figure modified from Tuchscherr et al. [335]

The sum of the conducted experiments emphasised the importance of different pathogenic bacterial strategies (s. Figure 15). Based on the fact that the low cytotoxic strains were clearly more successful in the applied infection models, the value of high cytotoxic strains within a bacterial population could be questioned, especially with regard to the high costs of toxin expression and the potential risk to kill the host and simultaneously eradicate the bacterial livelihood.

It would be helpful to address this issue from an ecological aspect. When analysing the relation of *S. aureus* and humans, painful and life-threatening infections understandably cause the most attention. However, in the majority of cases, *S. aureus* colonises the host without being noticed by the latter [43]. Therefore, the role of certain staphylococcal factors must always be considered in relation to the host environment. Once the bacterium is inside the hosts body, expressed toxins have a dramatic effect on the fate of the host as well as of the bacteria [4]. Still toxins recently have been shown to be beneficial for the stabilisation of host pathogen equilibrium.

The examples of PSMs, α-toxin and superantigen which were listed in the introduction, demonstrate the bifunctional nature of toxins, depending on the environment. Additionally, toxins have classical advantages like releasing host bound nutrients, killing immune cells and facilitating the penetration of deeper tissue structures [336]. For the tested models, the opposing strategy of low virulence was the better adapted one. This is supported by the often-cited avirulence theory which forecasts the stepwise co-evolution of parasites with their host towards total avirulence [337]. A practical evidence for this theory is the fact that low virulent *S. aureus* strains are able to survive better in human serum [302], which is a big fitness advantage in terms of the development of invasive diseases. Moreover, *agr*-defective strains are often detected in nosocomial infections [322, 338, 339] probably due to their metabolic advantage resulting from the reduced toxin expression [340]. The observation of staphylococcal evolution in real patients revealed the occurrence of loss-of-function mutations in an important virulence regulator, leading to mutants, causes fewer severe infections in early stages of disease but showed unchanged abilities of abscess formation and blood stream dissemination [341]. The avirulence theory with its actual proofs emphasises the importance of the recognition of low virulent strains as concerning variant of staphylococcal pathogens. According to that, low cytotoxic strains of this study originated from severely ill patients and were shown to have a higher ability for persistence.

After portraying the qualities of both bacterial virulence and avirulence strategies, the question about the reason for their coexistence arises. Evolution is creating the diversity of life driven by the motor of natural selection [342]. Darwin's theory did not lose any of its relevance over the decades. Aspects of his seminal studies can easily be applied on host-pathogen relationships. The human body provides a multiplicity of different ecological niches and *S. aureus* can colonise and infect most of them. Furthermore, the transition from colonisation to infection is a very complex process, depending on many host, bacterial, and environmental factors, and the manifestation of the infection can vary in a considerable way from superficial to life-threatening systemic infection. These different facets provide a glimpse of the intricate human-staphylococcal interplay, virtually demanding for a versatile, adaptable pathogen.

4.1 Future perspectives

The analysis of the patient groups revealed major group-specific differences. In order to compare the plain bacterial effect on the patients, data should be corrected for patient characteristics like age or comorbidities in future studies [287].

As discussed above, the microarray analysis provided a basic overview over the individual genotype. The application of whole genome sequencing would reveal more detailed information about the genetic background. It would allow the estimation of gene functionality, depending on mutations. Moreover, SNPs could be detected and connected to the clonal complexes and phenotype in infection models.

According to the observation made by Laabei *et al*. that low virulent strains are more successful in infections [302] due to their better survival in human serum, the strains used in this study should be tested for their survivability as mentioned in the publication. It could verify an important hypothesis and test the method for reproducibility which improves the general scientific quality in this field.

A better understanding of the connection between bacterial gene expression and pathogenesis would be achieved by RNA-seq. This method allows the exact determination of expression profiles during e.g. colonisation and infection. It would facilitate the identification of adaptation patterns. With the help of dual RNA-seq from pathogen and host, the interplay of the two opponents could be illuminated. It would also allow the comparison between different stages of infection and different tissues. Additionally, the role of the host or different hosts would be taken into account by using dual RNA-seq. The combination of an infection experiment of primary cell culture from different donors and RNA-seq is a promising approach on the way to the identification of individual host differences and general bacterial strategies.

Finally, it should be kept in mind that, amongst other limitations (size, metabolic rate, ROS levels, diet, etc.), mice are not natural hosts of *S. aureus* and are only rarely infected by it [343]. It is possible that a pathogen as well adapted as *S. aureus* would not display the same phenotype in an artificial host. Therefore, murine infection models can reproduce an infection in humans only in approximation. In favour of better transferability of experimentally generated results, complex organ models with human cells (e.g. biochips) should be used as a complementation to classical animal models. Especially the transition from colonisation to infection could be monitored more easily in this model [344]. Another possibility could be the usage of humanised mice as a compromise between a human cell culture based approach and an animal model [343].

Prospectively, *S. aureus* research should contribute to the comprehension of the complex interaction of bacterium and host on a general, and especially, on an individual level with regard to the improvement of infection therapy.

5. References

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6. Appendix

Table S 1 References for *S. aureus* colonisation rates at different parts of the human body

Interaction / infection	Reference
Colonisation	[22]
Meningitis	[345]
Sinusitis	[346]
Pneumonia	[345]
Endocarditis	[347]
Skin and soft tissue infections	[345]
Spondylodiscitis	[191]
Emesis	[348]
Sepsis	[169]
Diarrhoea	[348]
Cystitis	[349]
Toxic shock syndrome	[350]
Osteomyelitis	[178]
Septic arthritis	[351]
Diabetic foot infections	[352]

Table S 2 Complete results of DNA -microarray. A = ambiguous, N = negative, P = positive

		Nasal colonisation													Prosthesis infection												Haematogenous osteomyelitis														Sepsis										
Strain name		N1	N4	N6	N7	N8	N9	N10	N11	N12	N13	N14	N15	M01	M02	M03	M04	M05	M06	M07	Jüze47	Hapo37	Reju49	Hawa51	Hafa83	Hebl44	Hegl68	O1	O2	O3	O4	D01	D02	D03	Josc25	Edbr39	Chwa42	Rahe58	S01	S06	Kakl54	Mabö56	Udmi62	Reho41	Ankö60	Gen842	Hewi37	Ilun61
Clonal complex		CC030	CC006	CC25	CC45	CC398	CC005	CC398	CC15	CC5	CC45	CC030 (ST34/42)	CC045	CC005	CC030	CC008	CC007	CC006	CC008	CC005	CC005	CC007	CC007	CC007	CC008 (ST239)	CC022	CC121	CC025	CC015	CC022	CC015	CC006	CC045	CC022	CC008	CC022	CC101	CC001	CC022	CC045	CC015	CC045	CC045	CC022	CC008	CC001	CC22	CC5
STRAIN AFFILIATION		CC30-MSSA	CC6-MSSA	CC25-MSSA [PVL+]	CC45-MSSA	CC398-MSSA	CC5-MSSA	CC398-MSSA	CC15-MSSA	CC5-MSSA	CC45-MSSA	ST34-MSSA	CC45-MSSA	CC5-MSSA	CC30-MSSA	CC8-MSSA	CC7-MSSA	CC6-MSSA	CC8-MSSA-[ACME]	ST5/ST225-MRSA-II, Rhine-Hesse	CC5-MSSA	CC7-MSSA	CC7-MSSA	CC7-MSSA	ST239-MRSA-[III+ccrC], Ueno&Hiramatsu-type	CC22-MSSA	CC121-MSSA	CC25-MSSA	CC15-MSSA	CC22-MSSA	CC15-MSSA	CC6-MSSA	CC45-MSSA	CC22-MSSA	CC8-MSSA	CC22-MRSA-IV (rnbB-seq1), UK-EMRSA-15	CC101-MSSA	CC1-MSSA	CC22-MSSA	CC45-MSSA	CC15-MSSA	CC45-MSSA	CC45-MSSA	CC22-MSSA-[ccrAB4]	CC8-MSSA	CC1-MSSA	CC22-MSSA-[ccrAB4]	ST5/ST225-MRSA-II, Rhine-Hesse
Domain 1 of 23S-rRNA	Ribos. STAU	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
glyceraldehyde 3-phosphate dehydrogenase, locus 1	gapA	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
katalase A	katA	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
coagulase	CoA	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
thermostable extracellular nuclease	nuc1	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
staphylococcal protein A	spa	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
IgG-binding protein	sbi	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
staphylococcal accessory regulator A	sarA	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
histidine protein kinase, sae locus	saeS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
sensor protein	vraS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
accessory gene regulator allele I	agrI (total)	N	P	P	P	P	N	P	N	N	P	N	P	N	N	P	P	P	P	N	N	P	P	P	P	P	N	P	N	P	N	P	P	P	P	P	N	P	P	N	P	P	P	N	P	N	P	N	N
	agrB-I	N	P	P	P	P	N	P	N	N	P	N	P	N	N	P	P	P	P	N	N	P	P	P	P	P	A	P	N	P	N	P	P	P	P	P	N	P	P	N	P	P	P	N	P	N	P	N	N
	agrC-I	N	P	P	A	P	N	P	N	N	P	N	P	N	N	P	P	P	P	N	N	P	P	P	P	P	N	P	N	P	N	P	P	P	P	P	N	P	P	N	P	P	P	N	P	N	P	N	N
	agrD-I	N	P	P	P	P	N	P	N	N	P	N	P	N	N	P	P	P	P	N	N	P	P	P	P	P	N	P	N	P	N	P	P	P	P	P	N	P	P	N	P	P	P	N	P	N	P	N	N
accessory gene regulator allele II	agrII (total)	N	N	N	N	N	P	N	P	P	N	N	N	P	N	N	N	N	N	P	P	N	N	N	N	N	N	N	P	N	P	N	N	N	N	N	N	N	N	N	P	N	N	N	P	N	P	N	P
	agrB-II	N	N	N	N	N	P	N	P	P	N	N	N	P	N	N	N	N	N	P	P	N	N	N	N	N	N	N	P	N	P	N	N	N	N	N	N	N	N	N	P	N	N	N	P	N	P	N	P
	agrC-II	N	N	N	N	N	P	N	P	P	N	N	N	P	N	N	N	N	N	P	P	N	N	N	N	N	N	N	P	N	P	N	N	N	N	N	N	N	N	N	P	N	N	N	P	N	P	N	P
	agrD-II	N	N	N	N	N	P	N	P	P	N	N	N	P	N	N	N	N	N	P	P	N	N	N	N	N	N	N	P	N	P	N	N	N	N	N	N	N	N	N	P	N	N	N	P	N	P	N	P
accessory gene regulator allele III	agrIII (total)	P	N	N	N	N	N	N	N	N	N	P	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	P	N	N	N	P	N	P	N	N
	agrB-III	P	N	N	N	N	N	N	N	N	N	P	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	P	N	N	N	P	N	P	N	N
	agrC-III	P	N	N	N	N	N	N	N	N	N	P	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	P	N	N	N	P	N	P	N	N
	agrD-III	P	N	N	N	N	N	N	N	N	N	P	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	P	N	N	N	P	N	P	N	N
accessory gene regulator allele IV	agrIV (total)	N	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	A	A	A	A	P	N	N	N	N	A	N	A	N	A	A	N	N	N	N	N	A	A	N	N	N	N	N
	agrB-IV	N	A	P	N	N	N	N	N	N	N	N	N	N	N	P	A	A	P	N	N	P	P	P	P	P	P	N	A	N	P	N	P	P	P	P	N	P	N	N	N	N	P	P	N	P	N	N	N
	agrC-IV	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
haemolysin delta	hld	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
alternate penicillin binding protein 2, defining MRSA	mecA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	P	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	P
truncated signal transducer protein MecR1	delta_mecR	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	P	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	P
glycerophosphoryl diester phosphodiesterase, associated with mecA	ugpQ	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	P	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	P
cassette chromosome	ccrA-1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	ccrB-1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

recombinase genes A/B-1																																																
plasmin-sensitive surface protein	plsSCC (COL)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
hypothetical protein from SCCmec elements	Q9XB68-dcs	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	P
cassette chromosome recombinase genes A/B-2	ccrA-2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	P
	ccrB-2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	P
potassium-trans-locating ATPase A, chain 2	kdpA-SCC	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P
potassium-transporting ATPase B, chain 1	kdpB-SCC	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P
potassium-trans-locating ATPase C, chain 2	kdpC-SCC	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P
sensor kinase protein	kdpD-SCC	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P
KDP operon transcrip-tional regulatory protein	kdpE-SCC	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P
methicillin-resistance regulatory protein	mecI	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P
signal transducer protein MecR1	mecR	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P
homolog of xylose repressor, associated with SCCmec-elements	xylR	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P
cassette chromosome recombinase genes A/B-3	ccrA-3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	ccrB-3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
mercury resistance operon	merA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	merB	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
cassette chromosome recombinase genes "ccrAA" (hypothetical) and ccrC	ccrAA (MRSAZH47)_probe 1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	ccrAA (MRSAZH47)_probe 2	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	ccrC (85-2082)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
cassette chromosome recombinase genes A/B-4	ccrA-4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N
	ccrB-4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	P	N
SCCmec XI	mecC	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	blaZ-SCCmec XI	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
beta-lactamase	blaZ	P	P	P	N	N	N	N	P	P	P	P	N	N	N	P	N	P	P	P	N	P	N	P	P	N	P	N	N	P	N	N	N	P	P	P	N	P	P	P	P	P	N	P	P	P	P	P
beta lactamase repressor (inhibitor)	blaI	P	P	P	N	N	N	N	P	P	P	P	N	N	N	P	N	P	P	P	N	P	N	P	P	N	P	N	N	P	N	N	N	P	P	P	N	P	P	P	P	P	N	P	P	P	P	P
beta-lactamase regulatory protein	blaR	P	P	P	N	N	N	N	P	P	P	P	N	N	N	P	N	P	P	P	N	P	N	P	P	N	P	N	N	P	N	N	N	P	P	P	N	P	P	P	P	P	N	P	P	P	P	P
rRNA adenine N-6-methyl-transferase, erythromycin/ clindamycin resistance	ermA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	P
erythro-mycin/clindamycin resistance	ermB	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
erythro-mycin/clindamycin resistance	ermC	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Linco-samid-Nucleo-tidyltrans-ferase	linA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
energy-dependent efflux of erythro-mycin	msrA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
macrolide efflux protein A	mefA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
probable lysylphos-phatidyl-glycerol synthetase	mpbBM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
virginia-mycin A acetyltransferase	vatA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
acetyl-transferase inactivating streptogramin A	vatB	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ATP binding protein, streptogramin-A-resistance	vga	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	vgaA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

virginia-mycin B hydrolase	vgb	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
bifunctional enzyme Aac/Aph, gentamicin resistance	aacA-aphD	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
amino-glycoside adenyl-transferase,to bramycin resistance	aadD	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3'5'-aminog-lycoside phospho-transferase, neo-/ kanamycin resistance	aphA3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
strepto-thricine-acetyl-transferase	sat	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
dihydro-folate reductase type 1	dfrA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
fusidic acid resistence	far1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
hypothetical protein associated with fusidic acid resistance	Q6GD50 (fusC)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
mupirocin resistence protein	mupR	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
tetrazyklin-resistance	tetK	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N
tetracycline-resistance	tetM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
chloramphenicol acetyltransferase	cat	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	cat (pC221)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	cat (pc223)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	cat (pMC524)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	cat (pSBK203R)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
23S rRNA methyltransferase	cfr	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
chloramphenicol/florfenicol exporter	fexA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
metallothiol transferase	fosB	P	P	P	N	N	P	N	P	P	N	P	N	P	P	P	N	P	P	P	P	N	N	N	P	N	P	P	P	N	P	P	N	N	P	N	P	N	N	N	P	N	N	N	P	N	N	P
	fosB-plasmid	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
quaternary ammonium compound resistance protein A	qacA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
quaternary ammonium compound resistance protein C	qacC	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	qacC (cons)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	qacC (equine)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	qacC (SA5)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	qacC (Ssap)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	qacC (ST94)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Transport-/Effluxprotein	tetEfflux	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	N	P	P	P	N	P	N	P	P	N	P	P	P	P	N	P	P	N	P
vancomycin resistance gene	vanA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
vancomycin resistance gene from enterococci and Clostridium	vanB	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
teicoplanin resistance gene from enterococci	vanZ	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
toxic shock syndrome toxin 1	tst1 (consensus)	P	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	tst1 ("human" allele)	P	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	tst1 ("bovine" allele)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
enterotoxin A	entA	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	P	N	N	N	N	N	N	P	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N
enterotoxin A, allele from strain 320E	entA (320E)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
enterotoxin A, allele from strain N315 = entero-toxin P	entA (N315) / entP	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	P	N	N	N	N	P	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P
enterotoxin B	entB	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N
enterotoxin C	entC	N	N	N	P	N	N	N	N	N	P	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	P	N	N	N	N	N	N	N	N	N	P	N	N
enterotoxin D	entD	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	P	N	N	P
enterotoxin E	entE	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
enterotoxin G	entG	P	N	P	P	N	P	N	N	P	N	P	P	P	P	N	N	N	N	P	P	N	N	N	N	P	P	P	N	P	N	N	P	P	N	P	N	N	P	P	N	P	P	P	N	N	P	P
enterotoxin H	entH	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	P	N	N
enterotoxin I	entI	P	N	P	P	N	P	N	N	P	P	P	P	P	P	N	N	N	N	P	P	N	N	N	N	P	P	P	N	P	N	N	P	P	N	P	N	N	P	P	N	P	P	P	N	N	P	P
enterotoxin J	entJ	N	N	N	N	N	N	N	N	N	N	N	N	A	N	P	N	N	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	P	N	N	P
enterotoxin K	entK	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N
enterotoxin L	entL	N	N	N	P	N	N	N	N	N	P	N	P	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	P	N	N	N	N	N	N	N	N	N	P	N	N
enterotoxin M	entM	P	N	P	P	N	P	N	N	P	P	P	P	P	P	N	N	N	N	P	P	N	N	N	N	P	P	P	N	P	N	N	P	P	N	P	N	N	P	P	N	P	P	P	N	N	P	P
enterotoxin N	entN (cons)	P	N	P	P	N	P	N	N	P	P	P	P	P	P	N	N	N	N	P	P	N	N	N	N	P	P	P	N	P	N	N	P	P	N	P	N	N	P	P	N	P	P	P	N	N	P	P

	entN (other than RF122)	P	N	P	P	N	P	N	N	P	P	P	P	P	P	N	N	N	N	P	P	N	N	N	N	P	P	P	N	P	N	N	P	P	N	P	N	N	P	P	N	P	P	P	N	N	P	P
enterotoxin O	entO	P	N	P	P	N	P	N	N	P	P	P	P	P	P	N	N	N	N	P	P	N	N	N	N	P	P	P	N	P	N	N	P	P	N	P	N	N	P	P	N	P	P	P	N	N	P	P
egc cluster	egc (total)	P	N	P	P	N	P	N	N	P	P	P	P	P	P	N	N	N	N	P	P	N	N	N	N	P	P	P	N	P	N	N	P	P	N	P	N	N	P	P	N	P	P	P	N	N	P	P
enterotoxin Q	entQ	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N
enterotoxin R	entR	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	P	N	N	P
Enterotoxin U and/or Y	entU	P	N	P	P	N	P	N	N	P	P	P	P	P	P	N	N	N	N	P	P	N	N	N	N	P	P	P	N	P	N	N	P	P	N	P	N	N	P	P	N	P	P	P	N	N	P	P
enterotoxin-like protein ORF CM14	entCM14 probe1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	entCM14 probe2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
haemolysin gamma / leukocidin, component B	lukF	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
haemolysin gamma / leukocidin, component C	lukS	P	P	P	A	P	P	P	P	P	P	P	A	P	P	P	N	P	P	P	P	A	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P	A	A	P	P	P	P	P	P	A	P
	lukS (ST22+ST45)	N	N	P	P	P	P	P	P	P	P	A	P	A	N	A	P	N	A	N	P	P	P	P	P	P	P	N	N	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	A
haemolysin gamma, component A	hlgA	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Panton Valentine leukocidin F component	lukF-PV	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Panton Valentine leukocidin S component	lukS-PV	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
F component from hypothetical leukocidin from ruminants	lukF-PV (P83)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
S component from hypothetical leukocidin from ruminants	lukM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
leukocidin D component	lukD	N	P	P	N	N	P	N	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	N	P	P	P	N	P	P	N	N	P	N	P	P	N	N	P	N	N	N	P	P	N	P
leukocidin E component	lukE	N	P	P	N	N	P	N	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	N	P	P	P	N	P	P	N	N	P	N	P	P	N	N	P	N	N	N	P	P	N	P
leukocidin/ haemolysin toxin family protein	lukX	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
leukocidin/haemolysin toxin family protein	lukY	N	P	P	N	P	P	P	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	N	P	N	N	P	P	P	P	P
	lukY (ST30+ST45)	P	N	N	P	N	N	N	N	N	P	P	P	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	P	N	P	P	N	N	N	N	N
putative membrane protein	hl	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
haemolysin alpha	hla	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	A	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
putative membrane protein	hlIII (cons)	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	N	P	P	P	N	P	N	P	P	N	P	P	P	P	N	P	P	N	P
	hlIII (other than RF122)	P	P	P	N	P	P	P	P	P	N	P	N	P	P	P	P	P	P	P	P	P	P	P	P	N	N	P	P	N	P	P	N	N	P	N	P	P	N	N	P	N	N	N	P	P	N	P
haemolysin beta	hlb-probe 1	P	P	P	N	P	P	P	N	P	N	P	N	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	N	P	N	P	N	P	P	P	P	P	P	N	N	N	P	P	P	P	P
	hlb-probe 2	P	P	P	N	P	P	P	N	P	N	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	N	P	N	P	P	P	P	P	P	N	N	N	P	P	P	P	P
	hlb-probe 3	A	P	P	N	N	P	N	P	P	N	P	N	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	N	P	P	P	P	P	P	N	P	N	N	P	P	P	P
	un-truncated hlb	N	N	N	N	N	N	N	N	N	P	N	N	N	P	N	N	N	A	N	N	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
staphylo-kinase	sak	P	P	P	P	N	P	N	N	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	N	P	P	P	P	P	P	P	P	N	P	P	P	P	N	P	P
chemotaxis-inhibiting protein (CHIPS)	chp	P	N	P	P	P	N	P	P	N	N	P	P	P	P	N	N	N	N	P	N	N	N	N	N	P	N	P	P	P	P	N	P	P	N	P	N	N	P	P	P	P	P	P	N	N	P	P
Staphyl. Comple-ment inhibitor	scn	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
exfoliative toxin serotype A	etA	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
exfoliative toxin serotype B	etB	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
exfoliative toxin D	etD	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
epidermal cell differen-tiation inhibitor	edinA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
epidermal cell differen-tiation inhibitor B	edinB	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
epidermal cell differen-tiation inhibitor C	edinC	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ACME-locus	ACME (total)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ACME-locus	arcA-SCC	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ACME-locus: ornithincarb-amoyltrans-ferase	arcB-SCC	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ACME-locus: carbamat-kinase	arcC-SCC	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ACME-locus: arginine/orni-thine-antiporter	arcD-SCC	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
aureolysin	aur (cons)	P	P	P	N	P	P	P	P	P	N	P	A	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	A	P	P	A	P	P	P	P	P

	aur (Other than MRSA252)	N	P	P	N	N	P	N	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	N	N	P	P	P	N	P	P	N	N	P	N	P	P	N	N	P	N	N	N	P	P	N	P
	aur (MRSA252)	P	N	N	P	P	N	P	N	N	P	P	P	N	P	N	N	N	N	N	N	N	N	N	P	P	N	N	N	P	N	N	P	P	N	P	N	N	P	P	N	P	P	P	N	N	P	N
serin- protease A	splA	N	P	P	N	N	P	N	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	N	P	P	P	N	P	P	N	N	P	N	P	P	N	N	P	N	N	N	P	P	N	P
serin- protease B	splB	N	P	P	N	N	P	N	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	N	P	P	P	N	P	P	N	N	P	N	P	P	N	N	P	N	N	N	P	P	N	P
serin- protease E	splE	P	P	P	N	N	N	N	P	N	N	P	N	N	P	P	P	P	P	N	N	P	P	P	N	N	N	P	P	N	P	P	N	N	P	N	N	P	N	N	P	N	N	N	N	N	N	N
glutamylendopeptidase	sspA	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
staphopain B, protease	sspB	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
staphopain A (staphylopain A), protease	sspP (cons)	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	sspP (other than ST93)	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
staphyl. exotoxin-like protein	setC	N	P	P	A	P	P	P	P	P	P	N	A	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
staphylococcal superantigen-like protein 1	set6-var1_11	N	N	N	N	P	P	P	N	P	N	N	N	P	N	P	P	N	P	P	P	P	P	P	P	N	P	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	P	N	N	P
	set6-var2_11	P	N	P	P	N	N	N	P	N	P	P	P	N	P	N	N	N	N	N	N	N	N	N	N	P	N	P	N	P	P	N	P	P	N	P	P	P	P	P	A	P	P	P	N	P	P	N
	set6-var1_12	P	N	N	N	P	P	P	N	P	A	P	N	P	P	N	N	N	A	P	P	N	N	N	P	P	P	N	N	P	N	A	P	P	P	N	N	P	A	N	A	A	P	P	N	P	P
	set6-var2_12	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	P	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	N	N	N	N	N	N	N	P	N	N
	set6-var4_11	P	P	P	P	P	P	P	P	P	P	P	A	P	P	P	N	P	P	P	P	N	N	N	P	N	P	P	P	N	P	P	P	N	P	N	N	N	N	P	P	P	P	N	P	N	N	P
	ssl01-RF122	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	ssl01/set6 (COL)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	P	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	P	N	N	N
	ssl01/set6 (Mu50+N315)	N	N	N	N	P	P	P	N	P	N	N	N	P	N	A	N	N	A	P	P	N	N	N	A	N	P	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	A	N	N	P
	ssl01/set6 (MW2+MSSA476)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	N	N	N	N	N	N	N	P	N	N
	ssl01/set6 (MRSA252)	P	N	N	N	N	N	N	N	N	N	P	N	N	P	N	N	N	N	N	N	N	N	N	N	P	N	N	N	P	N	N	P	P	N	P	N	N	P	N	N	N	N	P	N	N	P	N
	ssl01/set6 (RF122)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	ssl01/set6 (other alleles)	N	P	P	P	N	N	N	P	N	P	N	P	N	N	N	P	P	N	N	N	P	P	P	N	N	N	P	P	N	P	P	N	N	N	N	N	N	N	N	P	P	P	P	N	N	N	N	N
staphylococcal superantigen-like protein 2	ssl02/set7	N	P	P	N	N	P	A	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	A	P	P	P	A	P	P	A	P	P	A	P	P	P	N	P	N	N	A	P	P	P	P
	ssl02/set7 (MRSA252)	P	N	N	P	P	A	P	N	A	P	P	P	A	P	N	N	N	N	A	A	N	A	A	N	P	N	N	N	P	N	A	P	A	A	P	N	A	A	P	N	P	P	P	A	A	A	N
staphylococcal superantigen-like protein 3	ssl03/set8_probe 1	N	P	P	N	N	P	N	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	N	P	P	P	N	P	P	N	N	P	N	P	P	N	N	P	N	N	N	P	P	N	P
	ssl03/set8_probe 2	N	P	P	N	N	P	N	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	N	P	P	P	N	P	P	N	N	P	N	P	P	N	N	P	N	N	N	P	P	N	P
	ssl03/set8 (MRSA252, SAR0424)	P	N	N	N	N	N	N	N	N	N	P	N	N	P	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N
staphylococcal superantigen-like protein 4	ssl04/set9	N	P	P	N	N	P	N	P	A	N	N	N	A	N	P	P	P	P	P	A	P	P	P	P	N	P	P	P	N	P	P	N	N	P	N	P	P	N	N	P	N	N	N	P	P	N	P
	ssl04/set9 (MRSA252, SAR0425)	P	N	N	P	P	N	P	N	N	P	P	P	N	P	N	N	N	N	N	N	N	N	N	N	P	N	N	N	P	N	N	P	P	N	P	N	A	P	P	N	P	P	P	N	N	P	N
staphylococcal superantigen-like protein 5	ssl05/set3_probe 1	N	P	P	N	N	P	N	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	N	P	P	P	N	P	P	N	P	N	N	P	P	P	P	P
	ssl05/set3 (RF122, probe-611)	N	P	A	N	N	A	N	P	A	N	N	N	A	N	A	N	P	A	N	A	N	N	N	A	N	P	P	P	N	P	A	N	N	A	N	A	A	N	N	P	N	N	N	A	A	N	N
	ssl05/set3_probe 2 (612)	N	A	P	N	N	P	N	A	P	N	N	N	P	N	P	P	A	P	P	P	P	P	P	P	N	A	A	A	N	A	P	N	N	P	N	P	P	N	N	A	N	N	N	P	P	N	P
	ssl05/set3 (MRSA252)	P	N	N	P	P	N	P	N	N	P	P	P	N	P	N	N	N	N	N	N	N	N	N	N	P	N	N	N	P	N	N	P	P	N	P	N	N	P	P	N	P	P	P	N	N	P	N
staphylococcal superantigen-like protein 6	ssl06/set21	N	N	N	N	N	N	N	P	N	N	N	N	N	N	P	P	N	P	N	N	P	P	P	P	N	P	N	P	N	P	N	N	N	P	N	N	P	N	N	P	N	N	N	P	P	N	N
	ssl06 (NCTC8325+MW2)	N	N	N	N	N	N	N	P	N	N	N	N	N	N	P	P	N	P	N	N	P	P	P	P	N	P	N	P	N	P	N	A	N	P	N	N	P	N	N	P	N	N	N	P	P	N	N
staphylococcal superantigen-like protein 7	ssl07/set1	N	P	P	N	A	P	A	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	A	P	P	P	N	P	P	N	A	P	A	P	P	A	N	P	N	N	A	P	P	A	P
	ssl07/set1 (MRSA252)	P	N	N	N	A	A	P	A	A	N	P	N	A	P	N	A	N	N	A	A	A	A	A	A	A	A	N	N	A	A	N	A	A	N	A	A	A	A	N	A	N	N	A	N	A	A	A
	ssl07/set1 (AF188836)	N	N	N	P	P	A	A	N	N	P	N	P	N	N	N	N	N	N	N	A	N	A	N	N	P	N	N	N	P	N	N	P	P	N	P	N	A	P	P	N	N	N	P	N	A	P	N
staphylococcal superantigen-like protein 8	ssl08/set12_probe 1	N	P	P	N	N	P	N	A	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	N	P	P	N	N	A	P	N	N	P	N	P	P	N	N	P	N	N	N	P	P	N	P
	ssl08/set12_probe 2	N	P	P	N	N	P	N	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	N	P	P	P	N	P	P	N	N	P	N	P	P	N	N	P	N	N	N	P	P	N	P
staphylococcal superantigen-like protein 9	ssl09/set5_probe 1	N	P	P	N	N	P	N	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	N	P	N	N	P	P	P	P	P
	ssl09/set5_probe 2	N	P	P	N	N	P	N	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	N	P	N	N	P	P	P	P	P
	ssl09/set5 (MRSA252)	P	N	N	P	P	N	P	N	N	P	P	P	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	P	N	P	P	N	N	N	N	N
staphylococcal superantigen-like protein 10	ssl10/set4	A	A	P	A	P	P	A	P	P	P	A	A	P	A	P	P	A	P	P	P	P	P	P	P	P	P	A	A	N	A	P	P	P	P	P	P	P	N	P	A	P	P	P	P	P	N	P
	ssl10 (RF122)	N	P	A	N	N	N	N	A	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	A	P	P	N	P	A	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N
	ssl10/set4 (MRSA252)	P	N	A	P	A	A	P	N	A	A	P	P	A	P	A	A	N	A	A	A	A	A	A	A	A	N	A	N	N	N	A	A	N	A	A	A	A	N	A	N	A	A	A	A	A	N	N
staphylococcal superantigene-like protein 11	ssl11/set2 (COL)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	P	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	P	N	N	N
	ssl11+set2(Mu50+N315)	N	P	N	N	N	P	N	N	P	N	N	N	P	N	N	N	P	N	P	P	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P
	ssl11+set2(MW2+MSSA476)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	P	N	N

	ssl11/set2 (MRSA252)	P	N	N	N	P	N	P	P	N	N	P	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	P	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N
staphylococcal exotoxin-like protein, second locus	setB3	N	P	P	N	N	P	N	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	N	P	P	P	N	P	P	N	N	P	N	P	P	N	P	N	N	N	P	P	N	P
	setB3 (MRSA252)	P	N	N	P	P	N	P	N	N	P	P	P	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	P	N	P	P	N	N	N	N	N
	setB2	N	P	P	N	N	P	N	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	N	P	P	P	N	P	P	N	N	P	N	P	P	N	N	P	N	N	N	P	P	N	P
	setB2 (MRSA252)	P	N	N	N	P	N	P	N	N	N	P	P	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	P	N	P	P	N	N	N	N	N
	setB1	P	P	P	N	P	P	P	P	P	N	P	A	P	N	P	P	P	P	P	P	P	P	P	P	N	P	P	P	N	P	P	A	N	P	N	P	P	N	N	P	N	N	N	P	P	N	P
Capsule type 1	cap 1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
capsular poly-saccharide synthesis enzyme	capH1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
O-antigen poly- merase	capJ1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
capsular poly-saccharide biosyn- thesis protein	capK1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Capsule type 5	cap 5	N	N	P	N	P	P	P	N	P	N	N	N	P	N	P	N	N	P	P	P	N	N	N	N	P	N	P	N	P	N	N	N	P	P	P	N	N	P	N	N	N	N	P	P	N	P	P
capsular poly-saccharide synthesis enzyme	capH5	N	N	P	N	P	P	P	N	P	N	N	N	P	N	P	N	N	P	P	P	N	N	N	N	P	N	P	N	P	N	N	N	P	P	P	N	N	P	N	N	N	N	P	P	N	P	P
O-antigen poly- merase	capJ5	N	N	P	N	P	P	P	N	P	N	N	N	P	N	P	N	N	P	P	P	N	N	N	N	P	N	P	N	P	N	N	N	P	P	P	N	N	P	N	N	N	N	P	P	N	P	P
capsular poly-saccharide biosyn- thesis protein	capK5	N	N	P	N	P	P	P	N	P	N	N	N	P	N	P	N	N	P	P	P	N	N	N	N	P	N	P	N	P	N	N	N	P	P	P	N	N	P	N	N	N	N	P	P	N	P	P
Capsule type 8	cap 8	P	P	N	P	N	N	N	P	N	P	P	P	N	P	N	P	P	N	N	N	P	P	P	P	N	P	N	P	N	P	P	P	N	N	N	P	P	N	P	P	P	P	N	N	P	N	N
capsular poly-saccharide synthesis enzyme	capH8	P	P	N	P	N	N	N	P	N	P	P	P	N	P	N	P	P	N	N	N	P	P	P	P	N	P	N	P	N	P	P	P	N	N	N	P	P	N	P	P	P	P	N	N	P	N	N
capsular poly-saccharide biosyn- thesis protein	capI8	P	P	N	P	N	N	N	P	N	P	P	P	N	P	N	P	P	N	N	N	P	P	P	P	N	P	N	P	N	P	P	P	N	N	N	P	P	N	P	P	P	P	N	N	P	N	N
O-antigen poly- merase	capJ8	P	P	N	P	N	N	N	P	N	P	P	P	N	P	N	P	P	N	N	N	P	P	P	P	N	P	N	P	N	P	P	P	N	N	N	P	P	N	P	P	P	P	N	N	P	N	N
capsular poly-saccharide biosyn- thesis protein	capK8	P	P	N	P	N	N	N	P	N	P	P	P	N	P	N	P	P	N	N	N	P	P	P	P	N	P	N	P	N	P	P	P	N	N	N	P	P	N	P	P	P	P	N	N	P	N	N
intercellular adhesion protein A	icaA	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
intercellular adhesion protein C	icaC	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
biofilm PIA synthesis protein D	icaD	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
surface protein involved in biofilm formation	bap	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
bone sialoprotein-binding protein	bbp	P	P	P	P	P	P	P	N	P	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	bbp (cons)	P	P	P	P	P	P	P	N	P	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	bbp (COL+MW2)	N	P	P	N	A	N	A	N	N	N	N	N	N	N	P	P	P	P	N	N	P	P	P	P	N	N	P	N	N	N	P	N	N	P	N	P	P	N	N	N	N	N	N	P	P	N	N
	bbp (MRSA252)	P	N	N	N	N	N	N	N	N	N	P	N	N	N	N	A	N	N	N	N	A	A	A	N	N	P	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	N
	bbp (Mu50)	N	N	A	N	A	P	A	N	P	N	N	N	P	N	N	N	N	A	P	P	N	N	N	A	P	N	A	P	N	P	A	A	P	A	P	N	A	N	A	P	A	A	P	A	A	N	P
	bbp (RF122)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	bbp (ST45)	N	N	N	P	P	N	P	N	N	P	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	P	N	P	P	N	N	N	N	N
clumping factor A	clfA	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	clfA (cons)	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	clfA (COL+RF122)	A	N	N	A	P	N	P	N	N	A	A	N	N	A	P	P	N	P	N	N	P	P	P	P	N	N	N	N	N	N	N	A	N	P	N	P	N	N	A	N	A	A	N	P	N	N	N
	clfA (MRSA252)	P	N	N	N	A	N	A	N	N	N	P	N	N	P	A	A	N	A	N	N	A	A	A	A	N	N	N	N	N	N	N	N	N	A	N	A	N	N	N	N	N	N	N	A	N	N	N
	clfA (Mu50+MW2)	N	P	P	P	A	P	A	P	P	P	N	P	P	N	A	A	P	A	P	P	A	A	A	A	P	P	P	P	P	P	P	P	P	A	P	A	P	P	P	P	P	P	P	A	P	P	P
clumping factor B	clfB	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	clfB (cons)	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	clfB (COL+Mu50)	N	N	N	N	N	P	N	N	P	N	N	N	P	N	P	N	N	P	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	P	N	N	P
	clfB (MW2)	N	N	N	P	P	N	P	P	N	P	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	P	N	P	N	N	N	N	P	N	P	P	P	P	N	N	P	N	N
	clfB (RF122)	N	N	P	A	A	N	A	A	N	A	A	A	N	N	N	N	N	N	N	N	N	P	P	P	P	P	N	A	N	A	P	A	P	N	P	P	A	N	A	A	A	A	P	N	A	N	N
collagen-binding adhesin	cna	P	P	N	P	P	N	P	N	N	P	N	P	N	P	N	N	P	N	N	N	N	N	N	P	P	P	N	N	P	N	P	P	P	N	P	N	P	P	P	N	P	P	P	N	P	P	N
cell wall associated fibronectin-binding protein	ebh (cons)	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	N	P	P	P	N	P	N	P	P	N	P	P	P	P	N	P	P	N	P
cell surface elastin binding protein	ebpS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ebpS_probe 612	P	P	P	N	N	P	N	P	P	N	P	N	P	P	P	P	A	P	P	P	P	P	P	P	P	N	P	A	A	P	P	N	P	P	P	P	P	P	N	P	N	N	P	P	P	P	P
	ebpS_probe 614	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ebpS (01-1111)	N	N	N	P	P	N	P	N	N	P	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	P	N	N	N	N	N	N	P	N	P	P	N	N	N	N	N
	ebpS (COL)	N	N	P	N	P	N	P	P	N	N	N	N	N	N	P	P	N	P	N	N	P	P	P	P	P	N	P	P	P	P	N	N	P	P	P	N	N	P	N	P	N	N	P	P	N	P	N
enolase	eno	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
fibrinogen binding protein (19 kDa)	fib	N	P	P	N	N	P	N	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	N	P	P	P	N	P	P	N	N	P	N	P	P	N	N	P	N	N	N	P	P	N	P
	fib (MRSA252)	P	N	N	P	P	N	P	N	N	P	P	P	N	P	N	N	N	N	N	N	N	N	N	N	P	N	N	N	P	N	N	P	P	N	P	N	N	P	P	N	P	P	P	N	N	P	N

fibronectin-binding protein A	fnbA	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	fnbA (cons)	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	fnbA (COL)	N	N	N	N	N	N	N	P	N	N	N	N	N	N	P	N	N	P	N	N	N	N	N	P	N	N	N	P	N	P	N	N	N	P	N	N	N	N	N	P	N	N	N	P	N	N	N
	fnbA (MRSA252)	P	P	N	N	N	N	N	N	N	N	P	N	N	P	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N
	fnbA (Mu50+MW2)	N	N	P	P	N	P	N	N	P	P	N	P	P	N	N	N	N	N	P	P	N	N	N	N	P	N	P	N	P	N	N	P	P	N	P	N	P	P	P	N	P	P	P	N	P	P	P
	fnbA (RF122)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
fibronectin-binding protein B	fnbB	N	P	P	P	P	P	P	P	P	P	N	P	P	N	P	P	P	P	P	P	P	P	P	N	P	N	P	P	N	P	P	N	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P
	fnbB (COL)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	P	N	N	N
	fnbB (COL+Mu50+MW2)	N	P	A	P	P	A	P	P	P	A	N	P	A	N	A	A	P	A	A	A	A	A	A	N	A	N	A	P	N	P	A	N	A	A	N	P	A	A	P	P	P	P	A	A	A	A	P
	fnbB (Mu50)	N	A	P	A	N	P	A	A	A	P	N	A	P	N	N	N	A	A	P	P	N	N	N	N	N	N	P	A	N	A	P	N	N	A	N	N	N	N	A	A	A	A	N	A	N	N	A
	fnbB (MW2)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	P	N	N	N	P	P	N	N	N	N	P	N	P	P	N
	fnbB (ST15)	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	A	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N
	fnbB (ST45-2)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	P	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Major histocompatibility complex class II analog protein (=Extracellular adherence protein, eap)	map	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	map (COL)	N	N	P	N	P	P	P	P	A	N	N	N	P	N	P	N	N	P	P	P	N	N	N	P	N	P	P	P	N	P	N	N	N	P	N	N	N	N	N	P	N	N	N	P	N	N	N
	map (MRSA252)	P	N	N	P	P	N	P	N	N	P	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	P	N	P	P	N	N	N	N	N
	map (Mu50+MW2)	A	P	P	N	N	P	A	P	P	N	N	N	P	N	N	P	P	N	P	P	P	P	P	N	P	P	P	P	P	P	P	N	P	N	P	P	P	P	N	P	N	N	P	N	P	P	P
Staphylococcus aureus surface protein G	sasG	N	P	N	N	N	P	N	P	P	N	N	N	P	N	P	N	P	P	P	P	N	N	N	P	P	N	N	P	P	P	P	N	P	P	P	N	P	P	N	P	N	N	P	P	P	P	P
	sasG (COL+Mu50)	N	N	N	N	N	P	N	N	P	N	N	N	P	N	P	N	N	P	P	P	N	N	N	P	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	P	N	N	P
	sasG (MW2)	A	P	N	N	N	N	N	P	N	N	N	A	N	N	N	N	P	N	A	N	N	N	N	N	P	N	N	P	P	P	P	N	P	N	P	N	P	P	N	P	N	N	P	N	P	P	N
	sasG (OtherThan252+122	A	P	N	N	N	P	N	P	P	N	N	A	P	N	P	N	P	P	P	P	N	N	N	P	P	N	N	P	P	P	P	N	P	P	P	N	P	P	N	P	N	N	P	P	P	P	P
Ser-Asp rich fibrinogen-/bone sialoprotein-binding protein C	sdrC	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	sdrC (cons)	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	sdrC (B1)	N	N	N	P	N	N	N	N	N	P	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	P	N	P	P	N	N	N	N	N
	sdrC (COL)	N	P	N	N	N	N	N	P	N	N	N	N	N	N	P	P	P	P	N	N	P	P	P	P	N	P	N	P	N	P	P	N	N	P	N	N	N	N	N	P	N	N	N	P	N	N	N
	sdrC (Mu50)	N	N	N	N	P	P	P	N	P	N	N	N	P	N	N	N	N	N	P	P	N	N	N	N	P	N	N	N	P	N	N	N	P	N	P	P	N	P	N	N	N	N	P	N	N	P	P
	sdrC (MW2+MRSA252+RF122)	N	N	P	N	P	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	A	N	N	N	N	P	N	N	N	N	N	N	P	N	N
	sdrC (OtherThan252+RF122)	N	P	P	N	N	P	N	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	N	P	P	P	P	P	P	N	P	N	N	P	P	P	P	P
Ser-Asp rich fibrinogen-/bone sialoprotein-binding protein D	sdrD	P	P	P	P	P	P	P	N	P	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	sdrD (cons)	P	P	P	P	P	P	P	N	P	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P
	sdrD (COL+MW2)	N	N	P	P	N	N	N	N	N	P	N	P	N	N	P	N	N	P	N	N	N	N	N	P	N	N	P	N	N	N	N	P	N	P	N	P	P	N	P	N	P	P	N	P	P	N	N
	sdrD (Mu50)	N	P	N	N	N	P	A	N	P	N	N	N	P	N	N	P	P	N	P	P	P	P	P	N	N	N	N	P	N	P	P	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	P
	sdrD (other)	P	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	P	N	P	N	N	P	N	N	N	N	P	N	N	P	N
van Willebrand factor binding protein	vwb	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	vwb (cons)	P	P	P	N	P	P	P	P	P	N	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	A	P	P	A	P	P	P	P	P
	vwb (COL+MW2)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	P	N	N	N	N	N	P	N	P	N	N	N	N	N	N	N	P	N	N	P	N	N	N	N	N	N	P	P	N	N
	vwb (MRSA252)	P	P	N	P	N	N	N	N	N	P	P	P	N	P	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	N	N	N	N	N	N	P	N	P	P	N	N	N	N	N
	vwb (Mu50)	N	N	N	N	N	P	N	P	P	N	N	N	P	N	N	N	N	N	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	P
	vwb (RF122)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	P	N	N	N	P	N	P	N	N	P	N	N	N	N	P	N	N	P	N
immunodominant antigen B	isaB	N	P	P	N	N	P	N	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	N	N	P	P	P	N	P	A	N	N	P	N	P	A	N	N	P	N	N	N	P	P	N	P
	isaB (MRSA252)	P	A	A	P	P	A	P	A	A	P	P	P	A	P	A	A	A	A	A	A	A	A	A	P	P	A	A	A	P	A	P	P	P	A	P	A	P	P	P	A	P	P	P	A	A	P	A
defensin resistance protein	mprF (COL+MW2)	A	P	A	N	N	A	N	P	A	N	A	N	A	A	P	P	P	P	A	A	P	P	P	P	A	P	A	P	A	P	P	A	A	P	A	A	P	A	N	P	A	N	A	P	P	A	A
	mprF (Mu50+252)	P	A	P	N	P	P	P	A	P	N	P	P	P	P	A	A	A	A	P	P	A	A	A	A	P	A	P	N	P	A	A	P	P	A	P	P	A	P	P	A	P	P	P	A	A	P	P
transferrin-binding protein	isdA (cons)	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	isdA (MRSA252)	P	N	N	P	P	N	P	N	N	P	P	P	N	P	N	N	N	N	A	N	N	N	N	N	A	N	N	P	N	P	N	P	N	N	A	P	A	N	P	N	P	P	N	N	N	N	N
	isdA (Other Than MRSA252)	N	P	P	N	N	P	N	P	P	N	N	N	P	N	P	P	P	P	P	P	P	P	P	P	P	P	N	P	N	P	A	P	P	P	N	P	P	N	P	N	N	P	P	P	P	P	P
hypothetical protein, similar to integral membrane protein LmrP	lmrP (OtherThanRF122)	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	lmrP (OtherThanRF122)	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	lmrP (RF122)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	lmrP (RF122)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
type I site-specific deoxyribo-nuclease subunit, 1st locus	hsdS1-RF122	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
type I site-specific deoxyribonuclease subunit, 2nd locus	hsdS2-ST5+ST8	N	N	N	N	N	P	N	N	P	N	N	N	P	N	P	N	N	P	P	P	N	N	N	P	P	P	N	N	P	N	N	N	P	P	P	P	N	P	N	N	N	N	P	P	N	P	P
	hsdS2-MW2+476	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	P	N	N
	hsdS2-RF122	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	hsdS2-MRSA252	P	N	N	P	N	N	N	N	N	P	P	P	N	P	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	P	N	P	P	N	N	N	N	N

type I site-specific deoxyribonuclease subunit, 3rd locus	hsdS3-AllOtherThanRF122+252	N	P	P	N	N	P	N	N	P	N	N	N	P	N	P	N	P	P	P	P	N	N	N	P	P	N	P	N	N	N	P	N	N	P	P	P	P	N	N	N	N	N	N	P	P	N	P
	hsdS3-ST8+ST1+RF122	N	P	N	N	N	N	N	N	N	N	N	N	N	N	P	N	P	P	N	N	N	N	N	P	N	N	N	N	N	N	P	N	N	P	N	N	P	N	N	N	N	N	N	P	P	N	N
	hsdS3-Mu50+N315	N	N	P	N	N	P	N	N	P	N	N	A	P	N	N	N	N	N	P	P	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P
	hsdS3-CC51+252	P	P	N	N	N	N	N	N	N	N	P	N	N	P	N	N	P	N	N	N	N	N	N	N	N	P	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	hsdS3-MRSA252	P	N	N	N	N	N	N	N	N	N	P	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
type I site-specific deoxyribonuclease subunit, unknown locus	hsdSx-CC25	P	P	P	N	N	P	P	N	P	N	P	N	P	P	P	N	P	P	P	P	N	A	N	P	P	P	P	N	A	N	P	P	P	P	P	P	P	P	A	A	A	A	P	P	P	P	P
	hsdSx-CC15	N	N	N	N	P	N	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	P	N	A	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N
	hsdSx-etd	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	P	P	P	N	N	N	P	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N
hypothetical protein, located next to serine protease operon	Q2FXC0	P	P	P	N	N	N	N	N	N	N	N	N	N	N	P	N	P	P	P	N	N	N	N	P	N	N	P	N	N	N	P	N	N	P	N	P	P	N	N	N	N	N	N	P	P	N	N
Unspecific efflux/trans-porter	Q2YUB3	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
hypothetical protein	Q7A4X2	P	N	P	P	N	P	N	N	P	N	P	P	P	P	P	P	P	N	P	P	P	P	P	N	P	P	P	N	P	N	N	P	P	N	P	N	N	P	P	N	P	P	P	N	N	P	P
hyaluronate lyase, first / second locus	hysA1 (MRSA252)	P	N	N	N	N	N	N	N	N	N	P	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N
	hysA1 (MRSA252+RF122) and/or hysA2 (cons)	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	hysA1 (MRSA252+RF122) and/or hysA2 (COL+USA300)	P	N	N	N	P	N	P	P	N	N	P	N	N	P	P	N	N	P	N	N	N	N	N	P	N	P	N	P	N	P	N	N	N	P	N	P	N	N	N	P	N	N	N	P	N	N	N
hyaluronate lyase, second locus	hysA2 (All Other Than MRSA252)	N	N	N	N	N	P	N	P	P	N	N	N	P	N	P	N	N	P	P	P	N	N	N	P	N	N	N	A	N	P	N	A	N	P	N	N	P	N	N	P	N	N	N	P	P	N	A
	hysA2 (COL+USA300+NCTC)	P	N	N	N	P	N	P	P	N	N	P	N	N	P	P	N	N	P	N	N	N	N	N	P	N	P	N	P	N	P	N	N	N	P	N	P	N	N	N	P	N	N	N	P	N	N	N
	hysA2 (All Other Than COL+USA300+NCTC)	P	P	P	P	N	P	N	P	P	P	P	A	P	P	N	P	P	N	P	P	P	P	P	N	P	N	P	P	P	P	P	P	P	N	P	P	P	P	P	P	P	P	P	N	P	P	P
	hysA2-AllOtherThan COL+USA300+NCTC	P	P	P	N	N	P	N	P	P	N	P	N	P	P	N	P	P	N	P	P	P	P	P	N	P	N	P	P	P	P	P	N	P	N	P	P	P	P	N	P	N	N	P	N	P	P	P
	hysA2 (MRSA252)	P	N	N	N	A	N	P	N	N	N	P	A	N	P	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	A	N	N	N	N

Table S 3 Correlation Matrix

			Generation time	Haemolysis	Cell death	Invasion	Bacterial load	*hla*	*agrA*	*rnaIII*	*psmα*
Spearman's rho	**Generation time**	Correlation Coefficient	1.000000	0.033370	-0.160190	0.110662	0.233531	-0.091708	-0.075685	-0.211572	-0.190661
		Sig. (2-tailed)		0.825746	0.287579	0.464080	0.118293	0.544410	0.617139	0.158099	0.204362
		N	46	46	46	46	46	46	46	46	46
	Haemolysis	Correlation Coefficient	0.033370	1.000000	0.365156*	-0.421845**	-0.400432**	0.383605**	0.276966	0.485908**	0.519704**
		Sig. (2-tailed)	0.825746		0.012586	0.003500	0.005824	0.008497	0.062404	0.000618	0.000214
		N	46	46	46	46	46	46	46	46	46
	Cell death	Correlation Coefficient	-0.160190	0.365156*	1.000000	-0.327978*	-0.332347*	0.457747**	0.194203	0.525008**	0.605797**
		Sig. (2-tailed)	0.287579	0.012586		0.026069	0.024032	0.001381	0.195924	0.000180	0.000008
		N	46	46	46	46	46	46	46	46	46
	Invasion	Correlation Coefficient	0.110662	-0.421845**	-0.327978*	1.000000	0.823584**	-0.187706	-0.248296	-0.595023**	-0.427704**
		Sig. (2-tailed)	0.464080	0.003500	0.026069		0.000000	0.211596	0.096142	0.000013	0.003028
		N	46	46	46	46	46	46	46	46	46
	Bacterial load	Correlation Coefficient	0.233531	-0.400432**	-0.332347*	0.823584**	1.000000	-0.165556	-0.260068	-0.481838**	-0.433611**
		Sig. (2-tailed)	0.118293	0.005824	0.024032	0.000000		0.271521	0.080898	0.000698	0.002609
		N	46	46	46	46	46	46	46	46	46
	hla	Correlation Coefficient	-0.091708	0.383605**	0.457747**	-0.187706	-0.165556	1.000000	0.322107*	0.357760*	0.583457**
		Sig. (2-tailed)	0.544410	0.008497	0.001381	0.211596	0.271521		0.029031	0.014644	0.000021
		N	46	46	46	46	46	46	46	46	46
	agrA	Correlation Coefficient	-0.075685	0.276966	0.194203	-0.248296	-0.260068	0.322107*	1.000000	0.547826**	0.327536*
		Sig. (2-tailed)	0.617139	0.062404	0.195924	0.096142	0.080898	0.029031		0.000081	0.026282
		N	46	46	46	46	46	46	46	46	46

	rnalII	Correlation Coefficient	-0.211572	0.485908**	0.525008**	-0.595023**	-0.481838**	0.357760*	0.547826**	1.000000	0.685970**
		Sig. (2-tailed)	0.158099	0.000618	0.000180	0.000013	0.000698	0.014644	0.000081		0.000000
		N	46	46	46	46	46	46	46	46	46
	psmα	Correlation Coefficient	-0.190661	0.519704**	0.605797**	-0.427704**	-0.433611**	0.583457**	0.327536*	0.685970**	1.000000
		Sig. (2-tailed)	0.204362	0.000214	0.000008	0.003028	0.002609	0.000021	0.026282	0.000000	
		N	46	46	46	46	46	46	46	46	46
*. Correlation is significant at the 0.05 level (2-tailed).											
**. Correlation is significant at the 0.01 level (2-tailed).											

6.1 Index of abbreviations

°C	degree Celsius
µ	growth rate
µg	microgram
µm	micrometre
a.k.a.	also known as
ADAM10	A Disintegrin and Metalloprotease 10
Agr	accessory gene regulator
AIP	autoinducing peptide
ALPL	alkaline phosphatase
AMP	antimicrobial peptide
ANOVA	analysis of variance
Bbp	bone sialoprotein binding protein
BHI	brain heart infusion
CC	clonal complex
CCL5 (a.k.a. RANTES)	Chemokine (C-C motif) ligand 5
cDNA	complementary DNA
CFU	colony forming unit
CHIPS	chemotaxis inhibitory protein
ClfA	clumping factor A
Cna	collagen adhesin
CoA	staphylocoagulase
DAMP	damage-associated molecular pattern
ddH_2O	double-distilled water
DIC	disseminated vascular coagulopathy
DMEM	Dulbecco's Modified Eagle Medium
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPBS	Dulbecco's phosphate-buffered saline
dUTP	desoxyuridine triphosphate
e.g.	*exempli gratia*, for example
Eap (a.k.a. Map)	extracellular adherence protein
ECM	extracellular matrix
EDIN	epidermal cell differentiation inhibitor
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
et al.	*et alii*
etc.	*et cetera*
FACS	fluorescence-activated cell scanning
FBS	foetal bovine serum
FITC	fluorescein isothiocyanate
FnBP	fibronectin binding protein
g	generation time
gDNA	genomic DNA
h	hour(s)
Hla	α-toxin, haemolysin α
HlgACB	γ-toxin
HUVEC	human umbilical vein endothelial cells
i.a.	*inter alia*
IF	interferon
IL	interleukin
isd	iron-regulated surface determinant
LTA	lipoteichoic acid

Luk	leukocidin
MHC	major-histocompatibility complex
min	minute(s)
ml	millilitre
mM	millimolar
mm	millimetre
MOI	multiplicity of infection
MRSA	methicillin-resistant *S. aureus*
MSCRAMM	microbial surface components recognizing adhesive matrix molecule
MSSA	methicillin-susceptible *S. aureus*
NET	neutrophil extracellular trap
nm	nanometre
NO	nitric oxide
NOD	nucleotide-binding oligomerization domain
OD	optical density
p.i.	*post infection*
PAMP	pathogen-associated molecular pattern
PCR	polymerase chain reaction
pHOB	primary human osteoblasts
PRR	pattern recognition receptor
PSM	Phenol soluble modulin
PVL	Panton-Valentin leukocidin
qSOFA	quick SOFA
RANTES (a.k.a CCL5)	regulated on activation, normal T cell expressed and secreted
RNA	ribonucleic acid
RNA-seq	RNA sequencing
ROS	reactive oxygen species
rpm	rounds per minute
s	second(s)
s.	see
S. aureus	Staphylococcus aureus
S. carnosus	*Staphylococcus carnosus*
sak	staphylokinase
SCIN	staphylococcal complement inhibitor
SCV	small colony variant
SERAM	secretable expanded repertoire adhesive molecules
SigB	sigma factor σB
SOFA	Sepsis-related Organ Failure Assessment
Spa	protein A
SSL	staphylococcal superantigen like protein
TF	tissue factor
TLR	toll-like receptor
TNF-α	tumour necrosis factor α
TRAIL	tumour necrosis factor-related apoptosis-inducing ligand
TSST	toxic shock syndrome toxin
U	unit(s)
vs.	*versus*
vWbp	von-Willebrand factor binding protein
wt/vol	weight / volume
WTA	wall teichoic acid
xg	gravitational acceleration
α-MEM	Minimum essential medium α

6.2 Index of figures

6.3 Index of tables

6.4 Curriculum vitae

6.5 Publications

6.5.1 Journal publications

Geraci, J., Neubauer, S., **Pöllath, C.**, Hansen, U., Rizzo, F., Krafft, C., ... & Löffler, B. (2017). The Staphylococcus aureus extracellular matrix protein (Emp) has a fibrous structure and binds to different extracellular matrices. *Scientific reports*, *7*(1), 13665.

Tuchscherr, L., **Pöllath, C.**, Siegmund, A., Deinhardt-Emmer, S., Hoerr, V., Svensson, C. M., ... & Löffler, B. (2019). Clinical *S. aureus* Isolates Vary in Their Virulence to Promote Adaptation to the Host. *Toxins*, *11*(3), 135.

6.5.2 Conference contributions (Poster)

Chlamydia Workshop, 16th - 18th March 2016, Freiburg (Germany)
Different host cell reactions to persistent infection of human fibroblasts with *C. trachomatis* and *S. aureus*
M. Baier, **C. Pöllath**, C. Grosse, L. Tuchscherr, B. Haschke, J. Rödel, K. Rennert, A. Mosig, U. Neugebauer, B. Löffler

5th Joint Conference of the DGHM & VAAM, 5th–8th March 2017, Würzburg (Germany)*
Staphylococcus aureus pathogenesis: from sepsis to hematogenous chronic bone infections.
C. Pöllath, L. Tuchscherr, B. Löffler

6th International Conference on Microbial Communication for Young Scientists, 20th – 23rd March 2017, Jena, Germany*
Staphylococcus aureus pathogenesis: from sepsis to hematogenous chronic bone infections.
C. Pöllath, L. Tuchscherr, B. Löffler

Sepsis Update Weimar 2017 – 8. Internationaler Kongress der Deutschen Sepsis-Gesellschaft, 06th – 08th September 2017, Weimar, Germany
In depth localization of *Staphylococcus aureus* in a hematogenous bone infection mouse model using two-photon microscopy.
C. Ebert, **C. Pöllath**, V. Hörr, Y. Ozegowski, L. Tuchscherr, B. Löffler, U. Neugebauer

70. Jahrestagung der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM) e. V., 19th - 21st February 2018, Bochum, Germany

The *Staphylococcus aureus* extracellular matrix protein (Emp) has a fibrous structure and binds to different extracellular matrices.

J. Geraci, S. Neubauer, **C. Pöllath**, U. Hansen, F. Rizzo, C. Krafft, M. Westermann, M. Hussain, G. Peters, M. W. Pletz, B. Löffler, O. Makarewicz, L. Tuchscherr

Staphylococcus aureus pathogenesis: from sepsis to hematogenous chronic bone infections. *****

C. Pöllath, L. Tuchscherr, B. Löffler

Focus on Microscopy, 14th-17th April 2019, London, UK

Localisation of Bacteria During Hematogeneous Osteomyelitis.

C. Ebert, A. Tannert, V. Hörr, C. Geppert, **C. Pöllath**, L. Tuchscherr, B.a Löffler, J. Popp, U. Glaser U. Neugebauer

***active presentation**

6.6 Statements

6.6.1 Selbständigkeitserklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und nur unter Verwendung der angegebenen Hilfsmittel und Literaturquellen angefertigt habe. Ich habe alle Dienstleistungen und Materialien, die ich von Dritten erhalten habe, ordnungsgemäß als solche gekennzeichnet.

Jena, den 03.11.2019

Christine Pöllath

6.6.2 Erklärung zur Dissertation

Hiermit erkläre ich, dass ich die vorliegende Dissertationsschrift noch nicht als Prüfungsarbeit für eine staatliche oder andere Prüfungen eingereicht habe. Des Weiteren versichere ich, dass ich bisher noch keinen Antrag zur Eröffnung des Promotionsverfahrens an einer anderen Hochschule eingereicht habe. Ich versichere, dass ich nicht die Hilfe eines Promotionsberaters in Anspruch genommen habe und dass Dritte keine geldwerten Leistungen für Arbeiten, die im Zusammenhang mit dem Inhalt dieser Dissertation stehen, von mir erhalten haben. Die geltende Promotionsordnung der Fakultät für Biowissenschaften der Friedrich-Schiller-Universität Jena ist mir bekannt.

Jena, den 03.11.2019

Christine Pöllath

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